

Aftermath of the Orlov trial

THE imprisonment last week of the physicist Yuri Orlov for the maximum possible term on charges of slandering the Soviet state in connection with his Helsinki Monitoring Group activities is yet another step—but a very large one—along the road that the Soviet Union is taking towards crushing those who dare to question the state's omniscient benevolence. Fortunately the scientific community has it in its power to ensure that every time the state acts to stamp out criticism, the criticism spreads even further. The Orlov trial is as serious a challenge to dissidence as there has been in recent years, but its outcome provides a major opportunity for the advancement of those basic causes of human rights for which dissidents fight. It all depends now, however, on western scientists—not those who are always rallying to the support of distressed Soviet colleagues, but those who until the present may have felt distaste for what is going on but who have not yet been prepared to express their feelings.

Before an excess of piety is unleashed, we should clearly recognise that many socialist countries would claim that their support for human rights was as good as, if not better than, that of capitalist countries. They would point out that there is more to human rights than just the so-called political ones: to go where you wish to, talk to whom you choose, say what you want to and so on. There are also economic and social rights: to be gainfully employed, to be protected from inflation, to have adequate health care; and, they would say, the record on these human rights by no means favours the capitalist world.

This is not a trivial point and much of the ritual tut-tutting to be heard in the West after events such as that of Orlov's trial shows no sign of recognising that there are other points of view. Where such a defence goes wrong, however, is in assuming that in support of economic and social rights the state, with all its massive power, may be justified in curtailing political rights. Certainly there is much to admire in a system which delivers certain minimum standards of living, but does this have to be at the cost of the ruthless repression of

those few who do not see everything around them as quite so rosy as it is portrayed?

Discreet words in ears and mild disapproval obliquely voiced is clearly proving inadequate as the Soviet Union flexes its muscles for more trials. In this issue of *Nature* Valentin Turchin raises the difficult problem of boycotts. He envisages the selective boycott of official links such as exchange agreements and conferences, and, it must be said, arguments against cutting such contacts are not as strong as they used to be. But whilst action on the official level begins to look a serious possibility, there is still much that can be done at an individual level.

There is, amongst Soviet scientists, much buried support for those victimised by the government. As one scientist with no record of public support for dissidents recently put it, when well away from prying ears, "there is in our people a strong religious streak, and many of us see dissidents as the modern equivalent of martyrs". There are counterparts in the West who are disturbed by what they hear but who have not really been called upon to make their views known. No longer can we have the luxury of disapproving thoughts without some form of action. There is amongst scientists so much informal traffic, exchange of correspondence, sending of preprints and reprints, that it is surely the duty of those who are concerned to use these channels to let their Soviet counterparts know of this concern, and thus, maybe, to encourage these counterparts to let their buried views rise nearer to the surface. More formal channels are often vulnerable (*Nature's* Soviet coverage, for instance, is something that our numerous Soviet readers are not always entrusted with by the authorities). The informal ones are almost impossible to eliminate.

Scientists occupy a specially favoured position in international relations. For one thing they can provide prestige, power and even economic growth. For another the very nature of science requires easy communication across national frontiers. For too long we have taken advantage of being global citizens without realising the potential therein for the advancement of liberalising ideas. □



Why you should boycott the Russians

A plea to western scientists
from exiled dissident **Valentin Turchin**

LAST February, on the first anniversary of the detention of Professor Yuri Orlov, a well-known Soviet physicist, in Moscow's Lefortovo Prison, I wrote a letter about his case to all physicists who are members of the US National Academy of Sciences (NAS) and to certain other physicists. I suggested that American scientists demand that they send a representative to Orlov's trial. If the Soviet authorities refused this request the scientists should boycott co-operation with the Soviet Union in physics until Orlov is released. I noted that Orlov is the first member of an academy of sciences in the Soviet Union to be arrested on political grounds since Stalin's death, and that the charge against him had recently been reclassified, so that instead of three years in prison he has now been sentenced to seven years of strict regime imprisonment plus five years in internal exile.

I have received positive answers to my letter from 70 physicists, and I am very thankful to them for taking to heart a Russian colleague's fate. They are, of course, a minority. I also received two important letters, which listed arguments against a boycott. One is from the President of the NAS, Dr Philip Handler; the other from three professors of MIT: Herman Feshbach, Francis E. Low and Victor F. Weisskopf. I believe a discussion of the questions they raise is timely and of general interest.

Feshbach *et al.* argue that "the Soviet physicists rather than the authorities will be punished by cutting off contacts." The implication that the authorities will not be punished by a boycott is wrong. The boycott I suggest would be directed against official relationships not against scientific communication. It would not impair the flow of scientific publications, by far the main source of information for Soviet scientists; its most immediate result would be to cancel a number of official Soviet-American exchanges. A foreign trip is a fairly sweet carrot in the Soviet policy of whip and carrot by which it creates an obedient group of scientific leaders. The authorities would not welcome the loss of this carrot. A boycott would also be a tremendous blow to government prestige back home. They would find it difficult to explain.

Now let us turn to the scientists. The boycott would be a punishment for only some of them. Soviet citizens are divided into two categories, *vyezdnnye* and *neveyezdnnye*. The boycott would punish those scientists belonging to the first category, who are allowed by the authorities (or rather by the KGB, who always have a veto) to go abroad and to take part in joint projects. Is this a very cruel and unjust punishment compared to Orlov's fate? When Orlov was arrested, not a single person from the scientific establishment in the Soviet Union showed the slightest concern about what was done to him. The most salient and immediate reason was because they did not want to lose their chances (actual or potential) of going abroad. I believe this behaviour forfeits one's moral right to foreign trips. Not that I seek to deprive these people of foreign trips to punish them, but I do not see why this side-effect should prevent us from taking resolute action on behalf of such persons as Yuri Orlov, Sergei Kovalev and others. Soviet scientists who understand this would not be offended; those who do not understand forfeit their right once more. The majority of the scientists, the *neveyezdnnye* who are not permitted foreign trips or who do not want to behave in the way necessary to earn such permission, will welcome the

boycott as an effort to rescue a prisoner of conscience. They will lose little, if anything.

My correspondents' second argument is that the boycott would hamper the visits of western scientists to the USSR, which are "important for actions in support of those who are being persecuted by the Soviet authorities but who have not as yet been formally charged". But a boycott on behalf of arrested scientists is the best, if not the only, way to protect those who are not yet arrested! Visits to Soviet scientists support them only to the extent that resolute actions may follow. Boycotting official Soviet ties does not mean severing personal ties with persecuted scientists. On the contrary, they should be strengthened. Exchange of letters and materials and help with publications are priceless. And after all, one can visit the Soviet Union as a tourist.

The third argument is that the boycott would impair the exchange of ideas and, in particular, those ideas which "may hopefully contribute toward a better and peaceful world". I think that socially important ideas are most often communicated through the printed word or other mass media, not through brief conversations. Westerners, when they are in the Soviet Union, prefer to "do as the Romans do" so there is no "exchange of behaviour" during official visits. They justify their behaviour by referring to their position as guests, and also by saying that "you must not mix science with politics" and that "after all, every nation has its own customs". Soviet citizens, when they visit the West and return, do not change a bit, with very rare exceptions. Only those who are sure not to change receive permission to travel.

Restriction of free communication is already the main principle of the Soviet rulers. You in the West accept this model without serious attempts to insist on your model. Did you ever reject a Soviet delegation to a conference because the brightest scientist you had invited had not received permission to attend on 'political' grounds, that is, most often for his love of free exchange of information? You accept and obey the Soviet model in all your relationships with the Soviets; thereby you help to perpetuate it.

Can you press the Soviet system at least to start changing the model? Yes, you can. But you have only one serious means of pressure: to threaten and exercise specific boycotts and refusals. In so doing, you still further diminish the exchange of information, and this looks paradoxical. But scientists in the 20th century are not surprised by paradoxes. The solution is simple: you would be reducing those forms of exchange which are most important for the rulers, not for the ruled. Orlov was arrested for giving publicity to information on violations of the Helsinki Accord. Other people in the Soviet Union are arrested for possessing books published in the West. Do you think you fight for freedom of information when you reject a boycott in order to have a conference in Soviet Georgia instead of, say, Norway, or to give Mr Gvishiani (Premier Kosygin's son-in-law) an opportunity to run a department in a joint institute in Austria?

Another argument against boycotts and other resolute action which turns up often, is the fragility of detente and the peril of a global nuclear war. In answer to my letter, Dr Handler wrote:

"Since our first formal protest to the Soviet Academy on behalf of Benjamin Levich, a protest that has been followed by so many others, I have understood that the time might come when the course of events or the compelling sense of justice might bring us to the point of terminating our programmes of exchange and cooperation with the Soviet Union in matters scientific."

Nevertheless, Dr Handler rejects the idea of a boycott on the Academy level at the present moment. He continues:

"Meanwhile, I have continued to hope, albeit with little confidence, that the Soviet leadership would learn that a great nation can easily tolerate dissent and discussion whereas repression must inevitably breed revolt. More importantly, I have recognised how fragile is detente . . . Unfortunately, the alternative to detente appears to be the vision of two nuclear-armed superpowers on a collision course, the ultimate consequences of which are simply too awful to contemplate. Given these unstable circumstances, formal interruption of our scientific relations could have the effect of throwing a switch from detente back to the cold war which is but a prelude to a hot one . . . Thus, whereas I view the situation of Orlov—like the circumstances of Kovalev, Scharansky, Ginzburg and the deteriorating personal circumstances of Sakharov—as a matter of the highest priority and deepest concern, I have not yet found the course of wisdom to be formal interruption of our scientific relationship although that possibility remains."

I am fully conscious of Dr Handler's responsibility. I hope that boycott on the level of individuals, laboratories or universities will be wide enough to become effective. I hope also that if this turns out to be insufficient, the NAS will step in—a possibility that is indicated in Dr Handler's letter. But I think that the fear of a direct link from scientific boycott to nuclear war is exaggerated.

The cause of strain and instability in relations between the two superpowers lies in the character of the Soviet regime. The rulers of the USSR implement a way of life which they call 'socialism', but which in fact is simply a totalitarian dictatorship by the Communist Party. The rulers claim that the system is the most democratic in the



Valentin Turchin

'If you see totalitarianism as a horrible threat to mankind and your mind rebels at what the Soviets are doing to Orlov and others, you will certainly not reject a boycott, although aware of its double-edged nature'

world and that they are 'true representatives' of the people. But they know that they are not. Therefore, they must justify their 'socialism' compared to western democracy; they must justify their firm hold on absolute power. Now, failing to create higher living standards, they have only one way to do that: to demonstrate that western democracy (which they equate with 'capitalism') does not work. This is why they subvert any constructive initiative on a world scale.

They are also obliged to behave as imperialists. The leadership carefully and insistently cultivates imperialistic feelings in the population, and not without success. For me, one of the greatest shocks in the 1968 invasion of Czechoslovakia was that the overwhelming majority of people in the USSR really welcomed the invasion. Soviet man unconsciously compensates for his abject personal position by feeling like part of a superpower.

Of course, Soviet leaders do not want nuclear war; they are not madmen. But, having a vested interest in international instability, they create conditions which may lead to nuclear war accidentally, without their explicit wish. This is the real danger. The world faces tremendous problems of hunger, overpopulation, environmental pollution, conflict between nations and conflict between poor and rich. Constructive peaceful solutions are desperately needed. But Soviet leaders consistently block such solutions and con-

tribute to conflicts. One can hardly doubt the Soviet role in the oil crisis, international terrorism, the Cuban presence in Africa and the like. A principal characteristic of catastrophes is that you cannot foresee them in exact terms; to prevent them you must remove their roots long before they occur. This way I believe that even partial successes in human rights are of more relevance to avoiding nuclear war (or other catastrophes of yet unknown nature) than formal treaties, which Soviet leaders sign and ignore.

In the final analysis, your attitude toward a boycott in human rights matters depends on your acceptance of totalitarian rule as a phenomenon. Suppose you declare a limited boycott, and in response, the Soviets declare a sweeping counter-boycott, as a result of which some scientists suffer. Are you to be blamed? Those who accept totalitarianism as a reality which is unpleasant but cannot be changed, will say yes, you 'provoked' them. Those who do not accept totalitarianism will say no, for only the Soviet authorities are responsible for the repressions in the Soviet Union. In the fight for human rights you cannot count on speedy results and lose your nerve when the Soviets refuse to give in. When the Soviets realise that from now on, punishment for transgressions will be a permanent factor which they cannot change by being stubborn, the desired results will ensue. After all, the Soviet Union needs the West much more than the West needs the Soviet Union. Returning to Orlov's case, if you see totalitarianism as a horrible threat to mankind, and your mind rebels at what the Soviets are doing to Orlov and others, you will certainly not reject a boycott, although aware of its double-edged nature. If you accept totalitarianism (at least for those strange guys with 'mysterious Slav souls'), you will see Orlov's case as a sad but natural matter, something in the nature of an automobile accident: the worse for Orlov.

Feshbach *et al.* call for a petition. But the effect on the Soviet authorities of verbal actions has been diminishing during the past few years. Earlier, the prestige of the Soviets in the West was relatively high, and Soviet officials feared that a loss of prestige would immediately result in diminished scientific and technological cooperation. Because of this, they were rather sensitive towards criticism from scientific circles. Now they have little left to lose in the way of prestige, and they realise that they have not suffered a loss of cooperation. So why fuss about petitions and criticisms? I am not saying that petitions have become useless, but they are becoming so. To stop this trend you must do something real. In Orlov's case, a petition, signed by 220 physicists (18 February 1977), has already been issued. The only visible result has been to increase the severity of the charge against Orlov.

I am distressed by similarities in the attitude of the average American and the average Soviet person towards totalitarianism. I see the same readiness to capitulate without fight. In discussions of boycotting Soviet physics over Orlov's case, the standard response has been: "Anyway, you will not find many who agree to boycott." This is similar to what the Soviet man in the street (and the well disposed KGB officer) says to dissidents: "You will not change it, anyway."

Yurii Orlov once told me the following story. He telephoned a well-known Soviet physicist, Academician V. L. Ginzburg, to arrange a meeting. He intended to discuss questions of physics, not of public life, so it had nothing to do with his dissident activities. But no sooner had Dr Ginzburg realised to whom he was speaking, than he hurried to warn: "But bear in mind: problems of the progress of mankind lie outside my interest." I am afraid now that when I telephone a well-known physicist in this country, no matter what my purpose, the first thing I am going to hear will be: "But bear in mind: I am not going to boycott Soviet physics over Orlov's case." □

Americans cancel visits in protest over Orlov

CONCERN for the fate of Dr Yuri Orlov among his scientific colleagues in the West continues to increase. Already a 20-person delegation from the US National Academy of Science has cancelled its visit to Moscow, in protest against the 12-year sentence imposed on Dr Orlov, and two other American scientists have also cancelled separate missions. In the UK, where the announcement of the verdict came on the eve of the routine biennial London meeting of the British-Soviet Joint Commission for collaboration in science and technology, reactions were equally sensitive. Among other gestures, a letter of protest, signed by some 70 Fellows of the Royal Society has been handed to the delegation.

Responding to foreign concern, the Soviet media have devoted unprecedented coverage to the Orlov case, both for home and foreign consumption. Not surprisingly, in view of the Soviet desire for continuing scientific contacts with the West, a number of these reports attempt to minimise his role as a scientist.

"Trained as a physicist, Orlov devoted himself exclusively in recent years to compiling tendentious, false information for foreign anti-Soviet publications and radio stations", said *Vechernyaya Moskva*. "In the past a physicist, Orlov abandoned his research work in order to engage in fabricating slanderous information . . . with the aim of undermining the Soviet system", reiterated *Moskovskaya Pravda*.

However, according to Orlov himself (in a curriculum vitae supplied to Amnesty International) his whole scientific career has been involved with matters of academic and personal freedom. Born shortly after the accession of Stalin, Yuri Orlov grew up an apparently orthodox follower of official doctrine, joining the Party in 1948, while a student at Moscow State University. On graduating in 1953, he began working at the Institute of Theoretical and Experimental Physics. In 1956, his first scientific paper appeared; significantly, this was in *Nuovo Cimento*. As Orlov himself explains: "This was at the very beginning of freer publication on subjects previously suppressed as 'secret', though they were not in actual fact secret at all". The same year, he represented his country at a conference in Geneva.

However, also in 1956, Orlov delivered a speech at a closed Party meeting in his Institute, calling for increased democratisation. This meeting was called to discuss the documents to be submitted to the 20th Party Con-

gress in October of that year. This was the Congress which inaugurated Khrushchev's policy of de-Stalinisation; yet, ironically, Orlov, whose views might well have been cited in defence of such a 'thaw', was dismissed from the Institute, expelled from the Party, and had his name struck out from scientific reports and reviews, since that name, according to officialdom, "brings disgrace to Soviet science". He was forbidden to defend his thesis, and was unable to obtain any scientific or teaching work in Moscow. Finally, he obtained a post in Armenia, working on nonlinear focussing electron accelerator technology.

Making the same point . . .

In Moscow

Irina Orlov speaking outside the court where her husband was on trial

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In London

John Macdonald putting the case for Orlov at the mock trial

Here, Orlov was able ("with some pressure") to defend his thesis, and later under an amnesty granted by Khrushchev in connection with the celebrations of 40 years of Soviet rule in Armenia, Orlov received back his clearance for 'secret' work. In 1968, he was elected a Corresponding-Member of the Academy of Sciences of the Armenian SSR.

This election, says Orlov, "proved to be a surprise for Moscow", and he became subject to increasing bureaucratic restrictions. In 1973, he returned to Moscow, under conditions which he does not specify, but which suggest a

certain pressure. After several months, he found a post at one of the institutes of the Soviet Academy of Sciences, but in 1974 was dismissed after writing a letter defending Academician Sakharov during the 'press campaign' against him.

During his active scientific life, Orlov published at least 25 papers on the theory and technology of synchrotrons and particle physics.

Following his dismissal, when publication through official channels became impossible, he became a regular participant in the 'Sunday Seminars' founded by Aleksandr Voronel for refusenik scientists, one of the very few Gentiles to do so. In 1974, when the seminar group attempted to hold an international symposium (which was frustrated by KGB intervention), Orlov submitted a paper on uncertainty effects, which proposed new variants of the equations of non-local field theory. According to reliable reports, even while in pre-trial custody last year, he completed two papers on symbolic logic and particle theory respectively — these, however, have apparently disappeared into the court files.

Last week John Macdonald, Q.C., who was briefed by Mrs Irina Orlova to defend her husband, on the grounds that no Soviet lawyer could offer an adequate defence, and who was not given a visa to travel to Moscow, presented the 'case for the defence'. With true legal wit, Mr Macdonald denied that this was a mock trial—on the contrary, he averred, "the mock-trial was in Moscow". The venue for the hearing—the Institute of Physics—was chosen deliberately to stress Orlov's professional affiliation, which the Soviet media seem bent on denying.

Support by scientific colleagues abroad has, on occasion, had considerable success—in 1970, a world-wide outcry from his fellow biologists effected the release of Zhores Medvedev from a Soviet mental hospital. Mr Macdonald is now preparing an appeal against the Orlov sentence, based on articles 342-343 of the Soviet criminal procedure, which provide for appeal in the case of "onesidedness or incompleteness of the enquiry", since witnesses "whose testimony has substantial significance for the case" were not called.

The support already shown, and perhaps to come, to Yuri Orlov by his fellow physicists and by the scientific community at large may well have a significant effect on the outcome of such an appeal.

Vera Rich

GMAG wants to stay on

BRITAIN'S representative and gentlemanly Genetic Manipulation Advisory Group wishes to be prolonged for a further term. The 19-member GMAG, established in December 1976 and containing 8 members appointed as scientific and medical experts, 5 able to represent the public interest, 4 nominated by the Trades Union Council to represent the interests of employees, and 2 to represent the interests of management, had by February 1978 provided advice on the control of potential hazards from over 100 recombinant DNA experiments. Last week it published its first annual report.

GMAG bears no legal liability for the advice it offers, its recommendations have no direct statutory force, and—unlike the National Institutes of Health in the US—it has no sanction of withholding grants to stimulate researchers to follow its guidelines. It survives in the UK under the broad umbrella of the Health and Safety at Work Act, which puts an obligation on employers to follow the best advice available to maintain the welfare of their employees.

In its 18 months, GMAG has had notification of 236 workers involved in recombinant DNA research, and received 102 proposals for experiments from 27 centres. GMAG has placed 31 of the experiments under category I of the Williams containment guidelines, 40 under category II, 27 under category III, and 4 under category IV. The levels, says GMAG in its report, range from "good microbiological laboratory practice (at level I) to practice appropriate for the handling of the most dangerous pathogens (at level IV)".

The category IV experiments involve: random nucleic acid from mammals (or birds) in an undisturbed vector-host system (1 proposal); random nucleic acid from viruses pathogenic to man in a disabled system (2 proposals); and an experiment on infectivity in animals in which the containment is necessary to keep out other infections, not to contain the experimental material.

GMAG members inspected the only potential category IV containment facility in Britain—at Porton Down Microbiological Research Establishment near Salisbury—last Friday and, according to its director, Dr R. J. C. Harris, gave it a clean bill of health. Dr Harris expects to receive GMAG's letter of approval this week. If it is forthcoming Dr Harris believes experiments at level IV could begin at Porton in July.

Seven laboratories have been approved for category III experiments: two in London (Imperial College and

the Imperial Cancer Research Fund Laboratory), and others in Edinburgh, Glasgow, Porton, and in industry (G. D. Searle and Co Ltd at High Wycombe and ICI at Runcorn).

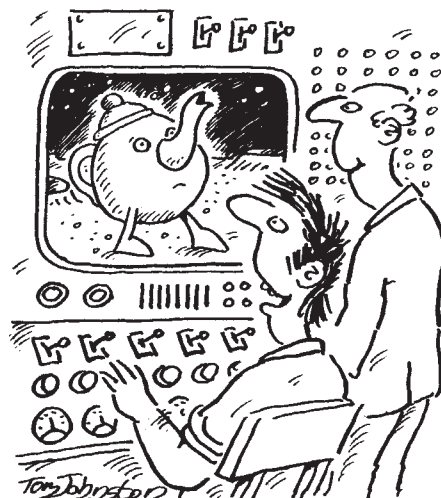
GMAG seems to be pleased with the speed with which it undertook the task allocated to it. It responded with definitive advice to the majority of proposals within 5 weeks of receipt. "The scientific community showed great public spirit in holding off an exciting field of research" says the report. "Any further delay . . . seemed likely to destroy [its] willingness to co-operate". Despite its haste, GMAG has experienced some resistance to its operations. In training biochemists and molecular biologists in the necessary microbiological safety techniques the group found that "even for very bright molecular biologists correct procedures are not instinctive". More disturbingly, they add "a small minority have made clear their disregard for these procedures".

The group offer a warning to the delinquent minority: "however strong an individual worker's intuitive feeling may be that the hazards have been greatly overestimated, we—and the public generally—have a right to expect of him, until we know more of the reality of the situation, that he should do the work either with scrupulous care or not at all".

GMAG nevertheless recognises the broad agreement in the field that the dangers were overestimated. "A substantial amount of recent work suggest that there is negligible risk of the laboratory strains of *E. coli* becoming converted into endemic pathogens or established in the alimentary canal" says the report. "If those findings stand up to careful scrutiny it will appear that many of the more extravagant predictions of risk can be disregarded".

However such a view rests as much upon feeling as on fact. The experiments which are commonly quoted as indicating that 'genetic engineering' goes on in nature as well as in the lab were undertaken in highly artificial conditions (cells doped with massive amounts of the necessary enzymes). And it appears that very recent work undertaken by Dr Harris at Porton indicates that the 'disabled' X 1766 strain of *E. coli*, the basis of the only NIH severely disabled (EK2) system, can be bred in a form which survives in the gut—so it may not be so disabled after all. It therefore follows that the continued scrutiny the report calls for, and the continued existence of GMAG, are necessary.

Robert Walgate



"I think Venus is going to be our kind of planet!"

Britain's giant leap to Venus

A PIECE of Britain left the Earth for Venus last week. "To the best of my knowledge", says Professor Houghton of Oxford University "it is the only piece of British hardware to leave the Earth's gravitational field". It went up on board the first of two missions to Venus, recently launched by the US National Aeronautics and Space Administration.

When the spacecraft reaches the planet in December, it will go into orbit and take measurements on the Venusian atmosphere. The pioneering piece of British hardware, a pressure modulator radiometer developed and built at Oxford, will be used by a joint US/British team to measure the temperature of the upper atmosphere and the distribution and structure of the higher clouds.

The second mission, scheduled for launch in August, is planned to arrive at Venus only six days after the orbiter. It will send several probes into the atmosphere to investigate the cloud content, the amount of radiation penetrating the upper atmosphere, and the nature of the Venusian surface. □

Space shuttle debut officially postponed

THE US National Aeronautics and Space Administration announced last week that the target date for the first orbital flight of the space shuttle has officially been changed from March to June of 1979, primarily because of problems encountered in the development of the vehicle's main engines. □

OSHA defends leap in carcinogen regulation

David Dickson describes a debate over US proposals for dealing with workplace carcinogens

IT WAS not quite the sell-out that had been predicted. Indeed the polite sparring that characterised the first day of public hearings in Washington last week on the US administration's new proposals for regulating occupational carcinogen was rather an anti-climax.

Yet the presence of ranks of lawyers alone was enough to indicate the wide potential impact—both national and international—of the proposals that have been put forward by the Department of Labor's Occupational Safety and Health Administration (OSHA).

For they are likely to provide the basis for dealing with known or suspected carcinogens across the complete spectrum of government agencies, including the Food and Drug Administration and the Environmental Protection Agency. The chemical industry claims that, as presently conceived, the proposals would add billions of dollars to the costs of production.

What OSHA is proposing is, in

effect, a quantum leap in the way that workplace carcinogens are identified, classified and regulated. At present this is done on a case-by-case basis, a thorough but laborious process which partly explains why, in its seven years of existence, OSHA has only developed regulations covering 20 of 1,500–2,000 suspected carcinogens.

Under the new proposals, all chemicals shown, under defined conditions, to cause cancer in experimental animals will be classified as carcinogenic. And the steps required to protect workers will be based not on any risk assessment or cost/benefit analysis, but on an assessment of what protective measures are felt to be economically and technically feasible.

The new proposals have been widely welcomed by many members of the scientific community—as well as trade unions and environmentalist groups—as an appropriate response to the current lack of precise knowledge

about the mechanisms or carcinogenicity, and to increasing evidence of the dangers of low levels of exposure which were previously considered safe.

Dr Arthur Upton, director of the National Cancer Institute and one of the scientific witnesses OSHA has asked to provide evidence at the public hearing, said last week that he believed the proposal "has a sound scientific basis and represents a prudent and justifiable approach to the identification and classification of chemical carcinogens". However, the proposals are being strongly contested by private industry, concerned at their potential economic impact.

Industry's attack is being spearheaded by the American Industrial Health Council, an ad hoc group established after the new proposals were announced by Labour Secretary Mr Ray Marshall last autumn. The AIHC now includes among its member 90 companies and 60 national trade associations, and is reputed to have raised over \$1 million to dispute OSHA's proposals. It has asked for

A summary of the proposals

All toxic substances shall be classified and subsequently regulated by the Secretary for Labor as follows:

Classification

Category I: All toxic substances that meet the definition of "potential occupational carcinogen"* in (i) humans, or (ii) two mammalian test species, or (iii) a single mammalian species, if those results have been replicated in the same species in another experiment, or (iv) a single mammalian species if those results are supported by short-term tests. Also any substance for which the Secretary considers there is sufficient evidence to classify as Category I.

Category II: Any substance defined as a "potential occupational carcinogen" in tests on animals or humans, but for which the evidence is considered only "suggestive". Also, any substance that meets such a definition in an unreplicated experiment in a single test species, and any other substance for which the Secretary considers there is sufficient evidence to classify as Category II.

Category III: Any toxic substance which is used in the US workplace, but which is not classified in Category I or Category II.

Category IV: Foreign toxic substances, ie, those not found in the US workplace.

*"Potential occupational carcinogen" means any toxic substance which (1) causes, at any level of exposure or dose, as a result of any oral, respiratory or dermal exposure, or any other exposure which results in the systemic distribution of the substance under consideration in the organism tested, an increased incidence of benign or malignant neoplasms, or a combination thereof, in (i) humans, or (ii) in one or more experimental mammalian species, or (2) in a statistically significant manner decreases the latency period between exposure and onset of neoplasm in (i) humans or (ii) in one or more experimental mammalian species.

Regulation

On classifying a substance as Category I, the Secretary shall immediately issue an Emergency Temporary Standard containing provisional exposure limits. Within 60 days, he or she shall issue a notice of proposed rule-making in order to establish permanent permissible exposure limits. These limits shall be set as low as feasible, and if suitable substitute materials are available, no occupational exposure shall be allowed.

Within 60 days of such classification, the Secretary shall issue a notice of proposed rule-making for permissible exposure limits. These would be (i) present OSHA standards, or (ii) where none exists, an appropriate level based on acute or chronic effects of exposure to the toxic substance other than carcinogenicity, or (iii) lower exposure levels if these are felt necessary. Further research would be stimulated to obtain additional data.

No exposure limits would be suggested, but information would be requested on whether substances should be considered for inclusion in Categories I or II.

No exposure limits would be suggested, unless information was subsequently received that the substance was used in the US workplace.

almost 20 hours to present evidence at the hearings and is bringing a number of scientists from Europe.

OSHA, emphasising the uncertainty that surrounds the interpretation of current scientific findings about carcinogenicity, has stated that, for the purposes of policy, it is basing its proposals on a number of propositions. The basic philosophy—a philosophy close to the heart of OSHA's director Dr Eula Bingham, until last year year professor of environmental health at the University of Cincinnati Medical School—is that where uncertainty exists, the prime concern is to protect the health of the worker.

Thus the administration claims that, given the lack of any conclusive evidence for a "threshold" below which carcinogenic effects can be ignored, any attempt at precise risk assessment at low level is unlikely to produce reliable results. It is therefore suggesting that exposure levels should be reduced "as low as feasible".

Similarly, OSHA states that because of the difficulties and limitations of human epidemiological studies—and the moral arguments against waiting for demonstrable proof of carcinogenicity from these studies—in general "as a practical rather than a theoretical matter, positive animal data should supersede negative human data".

Concerning the interpretation of evidence that a substance may cause only benign, rather than malignant, tumours, OSHA again comes down on the side of caution. It proposes "to place as much weight on an experiment in which only benign tumours are observed, as upon experiments in which both are induced."

In contrast to this approach, industry is arguing that the only "rational" basis for regulation should be firmly established scientific knowledge, which would include quantifiable parameters such as the relative potency of different carcinogens, and that such knowledge is a pre-requisite for regulation. Thus the lack of evidence for a threshold is interpreted as lack of evidence that no threshold exists, casting doubts on any regulatory principle based on the latter hypothesis. "No-effect levels have been repeatedly demonstrated for carcinogens in animals and man", claims Dr Hoerger of Dow Chemicals, chairman of AIHC's scientific committee.

And in questioning OSHA's proposal to give greater weighting to animal than human studies, the AIHC says that "since human epidemiological data are free from the uncertainties of extrapolation from animal tests, it seems quite unscientific not to use the data when available".

There are similar differences of approach over short-term tests, such as those for mutagenicity developed by

US firms accused of exporting health hazards

INCREASINGLY stringent control of hazardous production processes appears to be leading US companies to switch the work to countries with lower safety standards, particularly in the Third World.

In a number of industries, the banning of certain processes in the US has been followed by a significant increase in the import of materials made by these processes (in the case of benzidine dyes, for example, principally from Romania, Poland, India and France). And in other industries, American companies are shifting their own facilities elsewhere.

Some of the more dramatic examples of 'hazard export' have come from the asbestos industry. At least one study of the effects of lowering exposure levels to US workers has concluded that this is economically feasible provided that some of the more hazardous operations are conducted outside the US.

The import of asbestos textiles, for example, has risen dramatically in recent years from countries such as Mexico, Taiwan and Brazil as home

producers have experienced the need to meet lower levels of exposure.

Many of the production facilities are American-owned (although Japan owns plants in Taiwan and South Korea). And although most asbestos companies are careful to maintain the same safety standards at home and abroad, this is not always achieved.

Last year, for example, an environmental health scientist from Washington State University, Dr William M. Johnson, reported having found large quantities of loose asbestos both inside and outside a textile plant that had been opened by a large American-owned asbestos company in the Mexican border town of Agua Prieta in 1969.

Following the publicity surrounding his report, workers have been required to wear uniforms to cover street clothes, and the outside of the factory has been regularly cleaned. However, on revisiting the plant Dr Johnson claimed that the effects were mainly "cosmetic", and that the ground outside the factory on which children frequently played, for example, was still composed of 20% asbestos (see picture left).

Such situations are causing considerable concern to officials of the Occupational Safety and Health Administration (OSHA). "Cancer knows no national boundaries," says Dr Joseph Wagoner, head of carcinogens at OSHA.

US officials are uncertain about how to proceed. Working conditions outside the US are beyond the direct control of Washington; and many countries openly express a willingness to accept new jobs—however hazardous—because of unemployment problems.

David Dickson



Photographs taken by Dr Johnson outside Amatex asbestos textile plant, Agua Prieta, Mexico. March 1977.

Dr Bruce Ames of the University of California. Here the uncertainty arises from the fact that, although the correlation between the mutagenicity and the carcinogenicity of a substance is close, it is far from perfect.

OSHA, accepting this uncertainty as the grounds for suggesting that such tests should not be used as a sole basis for identifying carcinogenic potential, nevertheless feels they are sufficiently reliable to corroborate evidence of carcinogenicity gained from one animal test. Industry interprets this uncertainty in the opposite direction. Accepting that short-term tests have values as screening tools and as guides for more complete tests and studies, the AIHC adds that "the results of such tests are so unreliable as predictors of human response as to be

unsuitable as a basis for regulatory decisions".

Much of the public hearing which started last week, and will continue until the middle of July, is expected to hinge on such differences of interpretation of evidence in areas of scientific uncertainty. But in practice, although minor changes may be made to the details of OSHA's proposals, few expect any major shift in philosophy.

The real test, however, is likely to come later in the courts, where the issues will be determined not so much on scientific as on procedural ground. OSHA has already experienced difficulty here. Current moves to regulate benzene, for example, have come under heavy legal fire. The carcinogen proposals are one pudding whose proof will be the eating. □

Obtrusive energy



KENNETH MELLANBY

EVERYONE knows that the Earth's supplies of fossil fuels are finite, and that petroleum and natural gas, our most convenient energy sources, will be seriously depleted by the end of this century. The wisdom of replacing these by nuclear power, particularly the fast breeder reactor, is controversial. There is general agreement that natural renewable energy — solar radiation, wind, waves, the tides — should be more widely used, though there is disagreement about the magnitude of the contribution they will make. Conservationists are particularly enthusiastic, and perhaps over optimistic, about these natural sources, largely because they appear to be non-polluting and because it is imagined that they will not harm the environment.

This is partly true. Energy obtained directly or indirectly from the sun will not affect the heat balance of the globe, nor will it release large amounts of the gaseous oxides of carbon, sulphur or nitrogen. It does not add to the levels of radiation. But it may have other effects which are less acceptable than is commonly imagined.

The "wind bloweth where it listeth", but in many places it does so with sufficient consistency to supply energy which may be trapped by windmills. To most people a windmill is a picturesque erection of the kind formerly common in the fenlands of East Anglia and the Netherlands, now rarely seen unless maintained by the efforts and funds of preservationist groups. The modern windmill is very different; it is both more effective and more obtrusive. At the admirable UK National Centre for Alternative Technology near Machynlleth in Wales useful work on windpower is in progress. Various types of windmill of different sizes are being evaluated.

Unfortunately a large modern machine on a hill top is criticised by many local people who consider that it causes serious "visual pollution". If we wish to make a significant contribution (say 10%) to the power supply of any developed country then huge shiny erections will become a major feature of the landscape, with a greater impact than that made by the much-criticised pylons which now carry the cables of the electricity grid.

Solar power collection may be thought to be less obtrusive. Small panels on roofs are less pleasing to look at than thatch or eighteenth century pantiles, but they can be hidden or disguised. However, they can only make a minor contribution to single households. To collect solar energy on an industrial scale needs something very different. Although the total energy received by the earth from the sun is several thousand times that used by man's present activities, it is spread very diffusely even in the sunniest areas. Our present largest electrical generating stations, using coal, oil or nuclear energy, with an output of three megawatts, cover only a few acres and in some cases skilful landscape architects make them relatively unobtrusive. It is probable that at some future date improved techniques will enable solar energy of a comparable amount to be collected at an economic cost, but with the sort of efficiency that is suggested by present studies many square miles of collecting panels will be needed. Thus, for the United States, an area of collectors of over 50,000 square miles has been suggested to meet current use, and although this is under 2% of the total land area, their location will present problems. These will be even more serious in crowded countries like England. The situation might be expected to be easier in the deserts of the Sahara in Africa and in Australia, particularly suitable because of the intensity of the sunlight.

However, there would probably still be a very vocal opposition, led by many of those who are at present the most enthusiastic apostles of the use of solar energy. Thus in Australia today there is strong opposition to uranium mining, particularly from organised conservation groups. This is partly based on fears of the dangers of radiation, but also on environmental grounds. Even a proposed mining site of a few acres in a region of almost uninhabited desert is said to be of unique ecological importance, as well as being a sacred site of the aborigines. Solar panels covering thousands of square miles should surely be subject to similar objections.

Fast breeder delay would help proliferate weapons

ANY attempt to delay the development of the fast breeder nuclear reactor could increase, rather than decrease, the danger of the proliferation of nuclear weapons, according to a report by the Rockefeller Foundation.

The report is sharply critical of moves by President Carter to slow down the development of the fast breeder—in particular by terminating the Clinch River breeder project—on the grounds that the widespread use of plutonium would increase the likelihood of proliferation.

It argues that the United States should not only resume intensive development of the fast breeder, but also attempt to create a joint demonstration programme with other nations now committed to fast breeders.

The report, which was prepared by a Washington-based consulting firm International Energy Associates Limited, points out that most of these countries have already made the breeder part of their long-term energy plans. "Regardless of what the US decides, they are unlikely to alter or defer their own breeder programmes significantly", it says.

Any unilateral attempt by the US to delay breeder development could backfire by increasing the concern of other countries about potential shortages in fuel supply. This would lead to "more rapid and less considered deployment of breeders".

The report argues that a more realistic way of imposing control on breeder development would be to press for stricter international controls on the construction and placement of breeder fuel facilities. □

And then there were three

THE European Space Agency has taken the next step along the road to deciding who will be the sole European payload specialist aboard the first Spacelab mission in 1980. Last week it whittled down the number of candidates from four to three. The three to go on to the next stage of the decision-making process are Ulf Merbold (Germany), Claude Nicollier (Switzerland) and Wubbo Ockels (Netherlands). The final choice will not be made until just a few months before the flight. The unsuccessful candidates will have the consolation of working as ground-based specialists for the flight at NASA's Johnson Space Center. In the meantime, all three will become part of the Spacelab Payload Integration and Coordination in Europe (SPICE) team and undergo intensive training in Germany and the US. □



FIVE years ago, Torun University moved to a new campus in the suburb of Bielany, which thus became Poland's only "university village". In spite of this splendid new site, science at Torun, as elsewhere, is somewhat constrained by lack of finance. Never-

theless, some interesting research is going forward on a relatively shoestring budget—a tradition going back to the difficult days of the university's foundation in the aftermath of World War II.

Report by Vera Rich

Torun: local conditions provide new possibilities for research

ACCORDING to the official handbook of Torun University, the idea of a university for Pomerania (coastal Poland) has been advocated by its population since 1595. Not until the Nazi retreat of 1945, however, were any concrete plans put in motion.

Of the possible sites in the area, the other main contender, the former 'free city' of Gdansk, had suffered major devastation, and its famous Polytechnic Institute was virtually destroyed. Torun, however, being a quiet country town of little military importance and with strong historical ties with Germany had escaped the worst fury of the Third Reich.

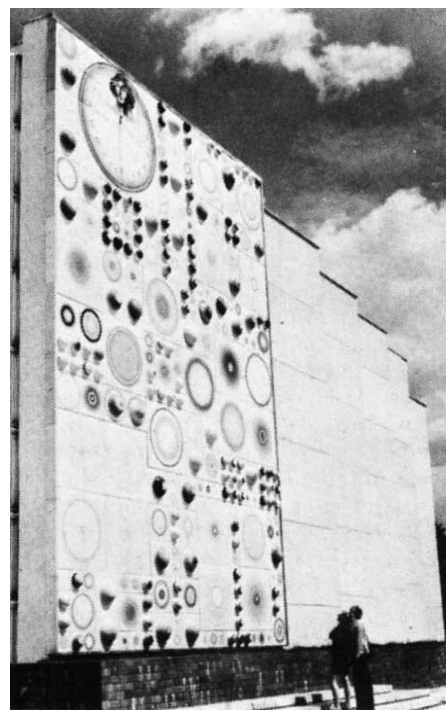
To any advocate of the Oxbridge or Ivy League tradition, Torun is, indeed, an ideal site of a university, a quiet township on the lower Vistula, whose old red brick and willow trees recall Hampton Court or Richmond. Moreover, Torun possessed the supreme advantage of having been the home-town of perhaps the most 'revolutionary' figure that Poland, that land of revolutions, ever produced. Accordingly, when the new university was formally established on 24 August, 1945, it was given the official title of the Nicolaus Copernicus University in Torun.

Staffing the new university caused few problems. The frontier changes which had restored most of Torun's catchment area from Germany to Poland had also metamorphosed the former Polish university cities of Wilno and Lwów into Lithuanian Vil'nyus and Ukrainian L'viv. Former staff of the Stefan Batory University in Wilno provided the nucleus for Torun,

augmented by a group from Lwów and a sprinkling from other Polish universities. In the case of physics, almost the whole former department from Wilno had disappeared during the war: only one remained, Professor Aleksander Jablonski, who, at the end of the war, found himself in Birmingham. Returning from England, he set about building up the physics institute virtually from scratch, and, by the time he retired had managed, in spite of a chronic lack of funds, to make the Torun school of physics world-famous in its own speciality — photoluminescence.

This particular field of research was chosen entirely pragmatically—Jablonski was himself an expert in photoluminescence; moreover, he knew that it is the cheapest field in which physicists may hope to do meaningful experimental work.

Financial difficulties, were, of course, most apparent in the early days, when, too, for a time (1951-56) there was the additional difficulty that national educational policy was geared to producing the professional cadres needed to rebuild the economy rather than education in the broader sense. Even now, however, financial controls still exist, particularly as regards imports of equipment from hard-currency areas. Although funds from the Polish government augmented by donations from academic and expatriate organisations throughout the world presented the university with a fine new campus in honour of the Copernicus quincentenary in 1973, the radio-astronomy department, for example, still lacks its promised second telescope which fell a victim to the world recession of the



late 1960s.

Yet Torun still manages to do valuable work. Integration of research and industry is, of course, a major topic of all science policy discussions throughout the Comecon block, and Torun can show some useful examples of this ranging from desalination to artificial fibre production. What is more unusual is the extent to which it has proved possible to use local conditions to provide new possibilities for research.

This is perhaps most clearly seen in the work of the Biological Institute. This comprises 14 sections, ranging from anthropology to microbiology. Some of the most interesting work, however, is specifically related to the Polish, and, in particular, the Pomeranian environment.

Limnology, for example, is an obvious subject for a team so near the thousands of Masurian lakes. Although pollution, inevitably, must be taken into account, the Torun scientists are not primarily concerned with ecological hazards, but with charting the whole process of eutrophication—the increase of levels of various salts (notably of nitrogen and phosphorus), the degradation and deterioration of water-bodies in the course of the ageing of the ecosystem. However, the limnological team have not been content merely to observe existing lakes—abundant as these are in the vicinity. Since 1959, a now-classic study on the Koronowo Dam reservoir has been seeking to establish the rules of ecological succession. Before the construction of the dam was begun in 1959, the ecology of the river Brda was thoroughly investigated. Since then, three papers have been published and the fourth is at

press, analysing the changing ecology of the lake over the years.

Another major field of biological research—plant hormones—is likewise related to local conditions. In Poland, as in all flat exposed regions, the lodging of wheat is a major cause of crop-loss. Since the use of fertilizers to improve yields also, normally, increases the stem length and, as a result, the likelihood of lodging, the use of growth-inhibiting hormones, notably CCC (chlorocholine chloride) was felt to be a valuable interim measure until reliable short-stemmed varieties of wheat are developed. Although, at present, there is some international concern about toxic side-effects of CCC, this is by no means a set-back to the Torun team. Over the years, their work on plant hormones has far outstripped the immediate needs of agriculture. Theoretical studies are in progress on the role of hormones, notably the gibberellins, in flowering. Another major project, supported by the US Department of Agriculture, is investigating the role of the gibberellins in the growth of pine trees. Torun has ready access to an abundance of pine-forests. Since working with trees is considerably more complicated than with small plants, not many biologists have been attracted into this particular sphere—hence the American interest in Torun. During the 15 years the project has been running, Dr Marian Michniewicz and his team have identified, extracted and purified a number of specific gibberellins in pine-trees.

Less happy, perhaps, is the position with research into sleep patterns and circadian rhythms. From the traditional subjects (rats, rabbits, hamsters), the project has progressed to birds—chaffinches, starlings and rooks, captured by means of drugged grain, although some attempts have also been made to breed in captivity. The effect of impoverished and enriched environments, especially in rats, is also under investigation. According to Dr Juliusz Narebski, although it is fairly easy to provide an impoverished environment, enriched conditions are somewhat harder to ensure. Ideally, he said, they should be working with open-field conditions and miniaturised telemetry as is being done in the USA and in France. This, however, Poland cannot afford, so they must, perforce, continue with laboratory work only.

The physicists, however, have no choice but to obtain equipment by some means or other. Even in the relatively cheap field of photoluminescence, it is no cheap matter to fit out a research laboratory, to say nothing of the needs of students. The Physics Institute, indeed, seems to have benefited less than most from the quincentenary—it is still housed in a some-



Radio astronomy at Torun: close cooperation with Cal Tech and Cambridge. Below: on campus at Poland's only university village.



what old-fashioned building in the town, and the main advantage of 1973 was that the chemists moved to the new campus, leaving the physicists a few additional laboratories. However, equipment has been found from somewhere—the new apparatus for decay curves in the subnanosecond region is being assembled from a veritable united nations of components, with dials in German, Czech, English and Russian. In the students' laboratories, there are the usual standard experiments on the luminescence of dyes, optical and magnetic resonance, the Franck-Hertz experiment and so on; here, much of the apparatus seems to have come from Zeiss of Jena. But, in the fourth year of the five-year course, in the words of Dr Ryszard Bauer, the students "begin to do science" as well as studying it, carrying out programmes of tests not to confirm text-book results but as part of the on-going work of the institute. This part of their work is done downstairs, in a laboratory less well-appointed than the teaching laboratories upstairs, with the typical Torun research array of 'international' components, linked into a working set-up by ingenuity and a few home-manufactured essentials. 'Doing science', as opposed to 'studying' it, in Torun, must surely throw a whole new light on the idea of international cooperation.

This international trend is even more apparent in the radio-astronomy department. Torun University possesses the largest astronomical observatory in Poland, and is doing valuable work both in the optical and radio fields. In optical astronomy, a spectral survey of 'peculiar' stars (novae, carbon stars, helium stars, variable stars) is in progress, a field of research especially associated with Dr Wilhelmina Iwanowska who retired recently.

The radio-astronomers are mostly concerned with the sun and the solar corona. Some interesting work has been done on the anomalies in the coronal scattering and refraction of stellar radio-emissions observed during the 1959 and 1970 solar maxima. Here, the Torun team work in close collaboration with Cal Tech and Cambridge—indeed, two of the team, Stanislaw Gorgolewski and Andrzej Kus actually trained in Cambridge under Sir Martin Ryle.

Cooperation stretches eastwards as well as west. When the Soviet Union offered to launch a Polish satellite as *Copernicus-500*, the Polish Academy of Sciences, who were officially responsible for the experiments, naturally approached Torun for suggestions. These were readily available; since the early 1960s Dr Gorgolewski had been advocating the use of a telemetry transmitter on a spacecraft—virtually a point source—to study the solar corona, and had suggested a similar experiment for the proposed Lunar International Laboratory that, in pre-recession days, was expected to succeed the Apollo programme. In the end, virtually all the apparatus aboard *Copernicus-500* was of Torun provenance.

Instrumentation is, indeed, one of Dr Gorgolewski's special interests. Currency restrictions and the very fact that radio-astronomy is a new science means that the institute has to design and construct the majority of the equipment it needs, although some vital components do come, often as gifts, from abroad. Not only does Dr Gorgolewski act as designer—he also closely supervises the whole construction process, down to the most ergonomic siting of a switch, and the measuring of the laminate housing. Thus a close involvement between scientist and instrument maker is established—and Dr Gorgolewski speaks no more proudly of his results in astrophysics than of the fact that his standard '19-inch' box for instruments has now become the Polish standard.

However practical and pragmatic the origin of this involvement, the result is an almost renaissance mutual interplay between the skills of scientist and craftsman, which rare as it is, seems particularly appropriate in a university named after Copernicus. □

news and views

Membrane movements and microfilaments

from Dennis Bray

IN recent years this subject has acquired the flavour of renaissance cosmology. There is the same abundance of speculative models and the same readiness to argue endlessly—cyclically—about a limited number of facts. There is, in addition, something of the early astronomers in the personality of the protagonists and we can identify a stubborn Galileo (*eppur si muove*) and many would-be Keplers. The only part so far lacking is that of Tycho Brahe to provide the definitive data, and even he may now have a counterpart.

The two principal phenomena to be explained are particle movements on migrating fibroblasts and antigen-capping on a lymphocyte. These two are almost certainly related and are generally considered to tell us something about how a cell moves. When a fibroblast crawls over the surface of the dish, particles of debris that the cell encounters are carried back on its dorsal surface as first described by Abercrombie and coworkers (*Expl Cell Res.* **62**, 389; 1970). They travel backwards faster than the cell advances and collect at a region near the centre of the flattened cell. A lymphocyte in suspension shows a similar clumping or 'capping' of surface antigens when treated with divalent antibodies (Taylor *et al.* *Nature new Biol.* **233**, 225; 1971). This reaction is energy dependent although there is an intermediate stage where small patches of cross-linked antigen form without metabolic energy. An important feature is that different antigens collect independently in response to their particular antibody.

Although it is commonly thought that these movements have something to do with cellular locomotion, attempts to account for them in terms of membrane proteins linked to actin and myosin (the usual mixture) have encountered difficulties. One of these is the apparent irreversibility of the backward movement of particles on

the surface which leaves no obvious way to restore the front end. Another is that, if we want to say that antigens cap because of an association with cellular actin, as was first suggested by de Petris and Raff (*Eur. J. Immun.* **2**, 523; 1972), then we have to accept that this link only occurs when the antigen is in an aggregated form—which at first sight requires a subtle and unprecedented mechanism.

It is in the attempt to meet these difficulties that the most radical solutions—so far—have been proposed. To provide for the return route of surface structures an earlier idea for amoeboid movement was resurrected. It was suggested that there was a cycle of membrane that travelled over the cell (faster on the free dorsal surface because no tension need be applied there) back through the cytoplasm to be finally reinstated at the leading edge into the plasma membrane (Abercrombie *et al. op. cit.*) This intriguing possibility fired the imagination of more ambitious spirits who added to it actomyosin networks, actomyosin arrows, actin on the inside, myosin on the outside, and other refinements until it reached an almost rococo elaborateness (see for example *CIBA Foundation Symposium* **14**, 1973). But for the present purposes we will keep to the straightforward original proposal. The comparable solution for capping is lipid flow: the elegant model proposed by Bretscher (*Nature* **260**, 21; 1976) which makes use of an artifice so far unprecedented in this area of biology known as the Mathematical Equation. The idea here is that the lipid component alone of the membrane cycles from front to back on the outside and in the reverse direction through the cytoplasm. Membrane proteins floating in this stream react according to their size, or more precisely to their diffusion coefficient, so that single proteins of moderate size are largely unaffected and remain distributed over the whole surface while large aggregates, such as are produced by antibody cross-linking, are swept along to the place where the

lipid alone disappears into the cell. Both this and the membrane-flow model have encountered difficulties since they were proposed: the lack of enough intracellular vesicles in electron micrographs is a count against both, while the failure of a fibroblast 'capped' with concanavalin A to rapidly replace these receptors is an argument against membrane flow (Vasiliev *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4085; 1970). None of these objections is fatal, especially if some flexibility is allowed in the interpretation of the basic models.

But there is more than one way to kill a cat, and neglect can be as effective as direct assault. A number of recent papers provide an insight into the mechanism of capping that makes the flow models seem less attractive. Several workers have found, by various fluorescent techniques, that the region of a lymphocyte that lies immediately beneath an antigen cap contains an accumulation of actin (Sundquist & Ernst *Nature* **264**, 226; 1976; Toh & Hard *Nature* **269**, 695; 1977) as well as myosin and tubulin (Schreiner *et al. J. exp. Med.* **145**, 1393; 1971; Gabbiani *Nature* **269**, 697; 1977). Caps are major deformations of the cell, and it could be said that they might collect cytoskeletal elements by secondary mechanisms, except that the same thing is true for patching (Bourguignon & Singer *Proc. natn. Acad. Sci. U.S.A.* **74**, 5031; 1977). Furthermore, Ash & Singer showed in 1976 (*Proc. natn. Acad. Sci. U.S.A.* **73**, 4575), that fluorescent concanavalin A on the surface of a fibroblast would go from an initially uniform distribution to one in which it was lined up with microfilament bundles within the cell provided that the concanavalin A was allowed to aggregate passively. This is a much more delicate reaction than overall clumping and, in a subsequent paper (Ash *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 5584; 1977) a similar effect was shown for three surface proteins in reaction with their specific antisera.

Finally, two papers in this issue

of *Nature* take the experiments away from the ultraviolet microscope and put them squarely on to the biochemical bench. On page 274 Koch and Smith describe a simple and effective technique by which actin can be precipitated from crude cell lysates by interaction with muscle myosin. They use this, together with other methods, to show that the microvilli shed from certain tumour cells are considerably enriched in the surface antigen H-2. A similar concentration of surface proteins on the microvilli of lymphocytes was recently detected by immunological methods (de Petris *Nature* 272, 66; 1978) and both seem to have interesting implications for the function of these important cellular extensions. More to the present point, in the second paper (page 278) Flanagan and Koch use the myosin-precipitation method to see how much surface immunoglobulin of a lymphocyte is associated with actin. The answer is, as one might hope, that much more is coupled to actin in extracts of cells that have been capped or patched than from cells with the unperturbed distribution. This provides a striking confirmation of the immunofluorescent work and shows, what was not strictly proven before, that the aggregation of surface proteins causes a new link to be made with actin.

Once again, these results do not disprove the ideas of membrane and lipid flow but by implication and counter-attraction they make them less plausible. For, if patches and caps are associated with cytoskeletal elements such as actin then they are unquestionably linked to the machinery of cellular locomotion. However difficult it might be to imagine mechanisms by which the small aggregates on the outside tell the actin on the inside to come hither, it really happens. In consequence, it seems inescapable that when patches move across the cellular surface they are in some way pulled by actomyosin—just as de Petris and Raff suggested. The only alternative is to say that membranous structures push the actomyosin and this has the logical status of a cart pushing a horse. Furthermore, if the recent views of amoeboid movement may be applied to vertebrate cells then any recycling that is to be done will probably be powered by actin—going perhaps between a polymerised and unpolymerised form. Whether this membrane protein, or that lipid molecule, follows the flow of actin through the cell or returns by lateral diffusion in the membrane then become questions which are still unanswered but which seem, suddenly, unimportant. □

plant with a potential pathogen. The importance and role of phytoalexins as natural plant 'antibiotics' is still uncertain, but their existence at least indicates an involvement in general, if not species-specific, resistance mechanisms.

The formation of phytoalexins in response to infection implies first, a mechanism whereby the plant recognises the invading organism as a potential pathogen; and second, the induction of the process of synthesis of the phytoalexin. In the past few years, the concept has developed that the plant has a recognition mechanism which enables it to recognise certain specific substances present either in the cell walls of the fungus, or exuded by the fungus. These substances, known as 'elicitors', are thus in an operational sense analogous to the antigens which are recognised by the immune system in animals, although there is no suggestion that the mechanism is similar or the effects as dramatic.

The first elicitor to be studied was a metabolite of the fungus *Monolinia fruticicola*, which was shown by I. A. M. Cruikshank and D. R. Perrin (*Life Sci.* 7, 449; 1969) to induce the formation of the phytoalexin phaseollin, in the bean *Phaseolus vulgaris*. Purification showed this substance, termed monolicolin A, to be a polypeptide of approximately 8,000 molecular weight. The best characterised elicitors have, however, been shown to be fungal wall components of a glucan nature. In an exemplary series of papers P. Albersheim and colleagues at the University of Colorado at Boulder characterised a class of wall components consisting of branched β -glucans with predominantly 3 and 3,6-linked glucosyl residues, present in the fungus *Phytophthora megasperma* var. *sojae*, which elicited the accumulation of the phenyl-propanoid phytoalexin glyceollin in soybeans (*Pl. Physiol.* 57, 751, 760, 766, 775; 1976). The elicitors were found both in the cell walls and in the culture filtrates, and consisted of a heterogeneous collection of polymers of macromolecular dimensions. Previously, A. J. Anderson-Prouty and Albersheim (*Pl. Physiol.* 56, 286; 1975) had isolated elicitors from the culture filtrate and cell walls of the fungus *Colletotrichum lindemuthianum* which induced phaseollin accumulation in beans; these were also glucans, with mainly 3 and 4-linked glucosyl residues and existed as polymers of around 1×10^6 – 5×10^6 molecular weight. Subsequently, a Russian group (Melitskii et al. *Dokl. Akad. Nauk. SSSR* 226, 1217; 1976; Chalova et al. *Dokl. Akad. Nauk. SSSR* 230, 722; 1976) showed that an elicitor could be obtained from the cell walls and mycelia of the potato pathogen *Phytophthora infestans*. This

Recognition and defence in plants

from H. Smith

PLANT diseases, pests and weeds cause the loss of an estimated 35% of crop products every year (Andreas Ber. *Landwirtschaft, Hamburg* 47, 2; 1969), and thus contribute indirectly, but substantially, to the suffering and starvation of a large proportion of the world population. Indeed, plant diseases may ultimately prove to be more important for the future of mankind than human diseases, but this conclusion would never be reached from a comparison of the investment in research into the two topics. Apart from the wholly commercial research on fungicides and pesticides carried out behind industrial closed doors, and in comparison to medical research, investment in fundamental plant pathology research in most Western countries is pitiful. Yet it is only through fundamental advances in our understanding

of the interaction between plants and microorganisms that we will be able to offer hope to the Third World, where the intensive application of chemicals is unlikely to become a practical proposition.

In spite of this unfavourable situation, a few brave and persistent individuals are beginning to make progress in some areas of fundamental importance, one being the mechanism whereby, in certain plants, the presence of a potential pathogen elicits a resistance response involving the synthesis of fungitoxic substances, known as phytoalexins, within the plant tissues. Phytoalexins usually possess antifungal activity against a wide range of fungi and are generally low molecular weight compounds, including flavonoids, isoflavonoids and isoprenoids. They have been reported in a wide variety of higher plants and their rates of synthesis are, characteristically, markedly enhanced by challenging the

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Do genes-in-pieces imply proteins-in-pieces?

from C. C. F. Blake

THERE has been some discussion in these columns recently of the significance and implications of the discovery that eukaryotic structural genes are interrupted by stretches of non-informational DNA. Gilbert (*News and Views* 271, 501; 1978) has suggested that this organisation could serve to speed evolution by providing mechanisms for the generation of novel proteins from parts of old ones. Subsequently Doolittle (*News and Views* 272, 581; 1978) has argued that the 'genes-in-pieces' structure is in fact a primitive form that was present in the genomes of the common ancestors of prokaryotes and eukaryotes.

These hypotheses are of great interest to those investigating protein structure because the structures of most larger proteins (molecular weight >20,000) also seem to be made up from 'pieces'. On the larger scale the pieces correspond to the independently-folded globular units called domains, although there are indications that domains may themselves be composed of smaller units called supersecondary structures (Rao & Rossmann *J. molec. Biol.* 76, 241; 1973). Although not enough is known about protein folding to rule out the possibility that domains are formed by some folding mechanisms particular to long polypeptide chains, so far no clear physicochemical explanation of their presence has been proposed. This leaves open the possibility of a specifically genetic origin, and in particular the question of whether they represent relics of the process by which the protein was

originally formed. Suggestions along these lines have been made (Rossmann *et al. Nature* 250, 194; 1974; Levine *et al. Nature* 271, 626; 1978) to account for the presence of domains of apparently similar structure and function in different enzymes.

In the light of these observations I wish to consider here the consequences of the rather obvious assumption that exonic regions of DNA (Gilbert has proposed that we use 'exon' for the stretches of structural DNA, and 'intron' for the interrupting stretches of non-informational DNA) correspond to integrally folded protein units—domains or supersecondary structures. If a new combination of exonic regions is to produce a novel protein molecule with new activity that confers advantage, then the new polypeptide chain must meet two conditions simultaneously. First, the chain must be able to fold into a stable globular form, as a necessary but not sufficient requirement for the second condition, namely an active site with new properties. If the new protein is made from random sections of old ones, the chances of meeting both conditions at the same time would undoubtedly be very small, perhaps vanishingly so. However, if the DNA was so organised that an exonic region corresponded to a folded protein fragment, then combinations would be much more likely to be stable through being the 'sum of parts', thus markedly improving the chances of meeting the dual requirements. If a protein built from two or more folded units maintains, as is most probable, the same folding pathways as in the original separated, or differently combined units, and, as also seems probable, subsequent point mutational events could not erase the resultant domain struc-

ture, it would remain as a permanent feature of the protein to be easily recognised when its structure was examined.

Furthermore in a new enzyme formed by the combination of two or more domains, the only region where the new constellations of amino-acid side-chains required to produce an active site with new properties could exist is specifically at the interfaces of the domains. All other regions on the new protein would contain the same patterns of amino acids as in the protein units from which they originated. In addition the domain interface could readily provide the surface depression or cleft that characterises active sites, and is an integral part of their activity. In every domain enzyme that I am aware of, the active site is in fact located precisely in the domain interface with each domain contributing to the cluster of amino acid side-chains that makes up the active site.

It can be seen that the evidence from protein structure is at least consistent with Gilbert's proposal that new protein molecules can be constructed from parts of pre-existing ones, and suggests the possibility that the parts, the transcripts of the exonic regions, are folded protein units. With Doolittle's extension of the time scale, necessary because many domain enzymes from prokaryotes and eukaryotes have homologous structures, there is now the possibility of a general mechanism for the formation of domain proteins that seem to be so common. Finally, it can be noted that the proposed correlation of exonic regions with folded protein units is open to test as soon as the eukaryotic DNA that codes for one of the known domain enzymes is sequenced. □

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also turned out to be a glucan and was active in inducing the formation of rishitin, a terpenoid phytoalexin, in the potato tissues.

The most recent development in this field is published by M. Stekoll and C. A. West of the University of California at Los Angeles in the January issue of *Plant Physiology* (61, 38; 1978) where they report the purification and characterisation of an elicitor from the culture filtrate of the fungus *Rhizopus stolonifer*. Although *R. stolonifer* could hardly be said to be a pathogen, in the strict sense, nevertheless the active substance induces the formation of a macrocyclic diterpene phytoalexin, known as casbene, in castor bean (*Ricinus communis*). Cas-

ebene was originally identified by West's group as being synthesised at relatively high rates in cell free extracts of castor bean seedlings which had previously been challenged by any one of a number of several fungi, including *R. stolonifer* and *Aspergillus niger* (Robinson & West *Biochemistry* 9, 70, 80; 1970; Sitton & West *Phytochemistry* 14, 1921; 1975). In the present very exhaustive study, Stekoll and West have developed a cell-free assay for casbene elicitor activity, in which split seedlings of castor bean are exposed to the test substances for a few hours, after which a cell-free extract is prepared and assayed for casbene synthetase; that is, the capacity to incorporate ¹⁴C-mevalonate into casbene. Chemical

characterisation and fractionation indicated that the elicitor is, in this case, a protein. On the other hand although substantial loss of elicitor activity occurred after Pronase treatment, periodate oxidation completely destroyed the capacity of purified fractions to stimulate casbene synthetase. The molecular weight of the active fraction on gel filtration was about 30,000 ± 5,000 and the authors conclude that the elicitor is a protein, and probably a glycoprotein.

Thus, two classes of elicitor seem to exist, one based on specifically branched glucan residues, which apparently do not contain any necessary protein, and one which is probably proteinaceous, but which requires car-

bohydrate for activity. Subsequent work of course might show similarities between the sugar residues of the casein elicitor and those of the glucan elicitors.

The question of how the elicitors are recognised by the plant is of obvious importance. In both the castor bean (Stekoll & West), and in cultured soybean cells (Ebel *et al.* *Pl. Physiol.* **57**, 775; 1976) exposure of the plants to elicitor results in a large increase in the extractable activity of enzymes responsible for the synthesis of the relevant phytoalexin. In the soybean cells, the activity of phenylalanine ammonia lyase, a critical enzyme in the biosynthesis of phenylpropanoids, increased many fold within 20 hours of the addition of purified elicitor, although similar but smaller increases were observed with a commercially available fungal wall glucan, nigeran, obtained from *Aspergillus niger*. It seems that the recognition mechanism, for the glucan elicitors is not very specific, especially since glucan fractions from several races of *Phytophthora megasperma* var. *sojae* all induce glyceollin formation in soybean seedlings even though the seedlings exhibit differential resistance to the various fungal races.

It may be, therefore, that the elicitor-phytoalexin system is not responsible for the extreme specificity shown by many resistance mechanisms. On the other hand, the discovery of a proteinaceous elicitor, with its inherently much greater potential for specificity, raises the possibility that specific elicitor recognition mechanisms may exist. For several years, antigenic cross reactivity between antibodies derived from soluble extracts of compatible host-parasite pairs has been reported, with generally little cross-reactivity being observed between non-compatible species. Recent work (see for example Palmerley & Callow *Physiol. Pl. Path.* **12**, 241; 1978) tends to the conclusion that such relationships obtained from studies of whole cell antigens do not account for the extreme host-pathogen specificity which is known to occur. On the other hand, there are indications from symbiotic relationships, for example the *Rhizobium*-root nodule association (Dazzo & Hubbell *Appl. Microbiol.* **30**, 1017; 1975) that cell surface antigens are responsible for specificity. In this case, common surface antigens on the *Rhizobium* and clover root hair cells, were proposed to be linked by a multivalent lectin, thus providing both specificity of recognition, and a mechanism for achieving intimate cell-to-cell contact. Speculating along these lines, and bearing in mind the capacity of lectins to bind specifically to carbohydrate residues, the possibility of fungal surface elicitors being

recognised by plant lectins seems feasible. A concentrated investigation of the antigenic relationships between fungal surface elicitors and plant surface proteins would seem to be a reasonable priority for future work. □

Fuel from magma

from Robert W. Cahn

SUCH modest amounts of geothermal energy as are currently extracted go to heat houses or make electricity, not very efficiently. The heat has of course to be used where it emerges. If a way could be found to store the heat chemically—to make a fuel—geothermal energy would at once become more widely useful. Such a way is proposed in a paper published from the Sandia Laboratories in New Mexico (Nortrup *et al.* *Int. J. Hydrogen Energy* **3**, 1; 1978).

The Sandia proposals are based on the partial reduction of water vapour at temperatures in the range 600–1300 °C in basaltic magma, with the simultaneous partial oxidation of FeO to Fe₂O₃. Basaltic lava typically contains ~10% of ferrous oxide and 1–2% of ferric oxide, partly dissolved in a silicate melt and partly as constituents of suspended solid particles. It has been shown in the geological literature that the oxygen fugacity of such lava can be simulated by certain solid oxide mixtures, such as magnetite-wuestite or nickel-nickel oxide, and the known thermodynamic characteristics of these simulant solids can be used to predict the equilibria in the proposed reduction of steam by lava: different simulant mixtures correspond to slightly different oxygen fugacities. Such calculations have been performed for a pressure of 1 kbar and compared with experimental measurements on lava at 950 °C (slightly below its solidus temperature), and good agreement established for a realistic value of oxygen fugacity. Under these conditions, the emerging gas contains 1–3% of hydrogen. These measurements refer to reactions with a low steam/basalt ratio, so that the basalt in effect buffers the steam: only a small fraction of the ferrous oxide is oxidised, and this is a necessary condition if acceptable hydrogen yields are to be achieved.

Taking this into account, it is estimated that some 2 million tonnes of hydrogen could in principle be recovered from water interaction with

1 km³ of high-temperature magma. The thermal loss arising from the need to heat the requisite amount of steam to the temperature of the magma would only cool the magma by about 50 degrees C.

The efficiency of this process could be enhanced by adding biological material, such as seaweed or cellulose, to the reacting water. This has previously been proposed on the basis purely of the heat content of magma (Poole & Williams *Bull. Atomic Scient.* **32**, 48; 1976), but without taking into account the available reducing potential. According to the new calculations, the hydrogen content could be enhanced and useful quantities of methane or carbon monoxide generated, depending on the reaction temperature.

The authors go on to consider practicalities. Plainly such a process would need to be concentrated either at mid-oceanic ridges (it is rather quaintly pointed out that sargasso weed is available conveniently for the mid-Atlantic ridge!) or along island arcs or circum-oceanic volcanic belts. The basaltic magma below mid-ocean ridges has high FeO content and high temperature and is available at a shallow depth, and the authors cite geological evidence for the availability of large coherent bodies of magma: a session on the search for such bodies formed part of the 1976 Fall Meeting of the American Geophysical Union. The practical problems in tapping such bodies, assuring intimate contact of the reagents and recovering the reduced gas mixture are immense, and the authors outline a few of the needed lines of research. □

Electric Universe

from P. C. W. Davies

GRAVITY is the force that controls the stars and galaxies. Although about forty powers of ten weaker than electricity, the cumulative effect of gravity in a massive body of astronomical size far outweighs electromagnetic forces, which tend to be self-neutralising. The reason for this is that all matter attracts gravitationally, but can both attract and repel electrically. Electrically charged matter therefore tends to accumulate charges of the opposite sign and achieve neutrality. This is the condition of ordinary matter.

Suppose, however, that a body is very hot, so that its atoms are dissociated into positive ions and electrons. Near thermal equilibrium the

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heat energy is shared more or less equally between the ions and electrons. But the electrons are thousands of times lighter than the ions, so they move dozens of times faster for the same quantity of energy. When this happens in a gravitating body like the Sun it leads to a curious effect. The rapidly-moving electrons are endowed with a sufficient velocity that they can actually fly away from the solar surface and escape the Sun's gravity altogether. Thus, the heat of the Sun simply boils off these highly agitated, mobile particles, leaving the more ponderous ions relatively undisturbed. Of course, the process does not go on for ever. When the electron abundance is somewhat depleted, the Sun acquires a net positive electric charge, whose electric field exerts an additional restraining force on the would-be escapees.

The fact that a star acquires an electric field through the above mechanism has been known at least since Sir Arthur Eddington treated the problem in 1962. In a recent paper in *The Astrophysical Journal* (220, 743; 1978) John Bally and E. R. Harrison of the University of Massachusetts have re-investigated this intriguing phenomenon. They pay special attention to the question of what happens to the expelled electrons. Previously it was supposed that they would form a cloud around the gravitating body and therefore screen its positive charge, but Bally and Harrison argue that this would not happen unless the body is surrounded by a perfect vacuum. In the real Universe, stars like the Sun do not have a well-defined surface, but possess an atmosphere that gradually merges into the tenuous interstellar medium. From a simple calculation the authors assert that the electron expulsion mechanism apparently works even in these interstellar regions. Thus, they argue that not merely individual stars, but entire galaxies—indeed galactic clusters—are electrically charged. The boiled off electrons have nowhere to go except the vast chasms of intergalactic space, which therefore accumulates a negative charge density.

In spite of its Velikovskian flavour, the Bally-Harrison electric universe unfortunately does not lead to any obviously important astrophysical consequences. They calculate that the average charge per mass is around 100 coulombs per solar mass, and the internal electric field of the Sun leads to a centre-to-surface potential difference of a mere 10^3 V (compare 10^9 V in the Earth's atmosphere), and about the same for a galactic cluster.

One effect of the electric field concerns rotation. A rotating charged body generates a magnetic moment, which in the case under discussion is given by the formula $\beta G^{\frac{1}{2}} J/c$ where G is Newton's gravitational constant, J is the angular momentum, c is the speed of light and β is a numerical constant. Oddly enough, such a formula was conjectured by P. M. S. Blackett in 1947, with β of the order unity. However, Bally and Harrison find $\beta \approx 10^{-18}$ in their model, for which typical magnetic fields work out at the absurdly small value of 10^{-16} gauss for the Sun and 10^{-23} gauss for the Galaxy. Such fields are completely swamped by other more powerful fields due to quite different mechanisms.

The authors conclude that, though intriguing, the physical significance of an electrically polarised universe is surprisingly obscure. □

Methotrexate resistance by gene amplification

from Robert Shields

THE mammalian genome has yet more shocks in store for molecular biologists. Until now it was thought that protein synthesis was regulated exclusively at the level of transcription of the gene or translation of the mRNA into protein. However, a recent paper from Robert Schimke's laboratory shows that some mammalian cells can regulate protein synthesis by way of selective gene amplification (Alt *et al. J. biol. Chem.* 253, 1357; 1978), a phenomenon already known in bacteria.

The system under study is the acquisition by cells of resistance to the drug methotrexate. This compound is a folic acid analogue and inhibits the enzyme dihydrofolate reductase which has an essential role in the biosynthesis of purines. Because purines are essential for cell survival methotrexate is widely used as a cancer chemotherapeutic agent, although unfortunately it is becoming clear that cells frequently become resistant to the drug.

In principle, methotrexate resistance can arise in a number of ways; alterations of cell permeability, alterations of the affinity of the enzyme for the drug or increase in the amount of enzyme present in the cell. There are in fact reports of resistance occurring as a result of all three mechanisms (Niethammer & Jackson *Eur. J. Cancer* 11, 845; 1975) but the one studied by the Stanford group is the approximately

200-fold increase in enzyme levels in certain resistant cells. No one will be surprised to learn that this increase is a consequence of increased levels of dihydrofolate reductase mRNA but what is surprising is that this increase is due in turn to a 200-fold amplification of the dihydrofolate reductase gene. This is unusual as the mechanism of increased gene expression (for instance after hormone treatment) has been examined in many cases and the number of genes for the affected proteins has remained at one per haploid genome.

Cells resistant to methotrexate were produced by prolonged culture in medium containing increasing concentrations of the drug. What happens when the drug is removed depends on the cell line examined. Quite often the resistance is stable and levels of dihydrofolate reductase remain high. However, in the cell lines looked at in some detail resistance is unstable and loss of resistance is accompanied by decreased dihydrofolate reductase levels (Alt *et al. op. cit.*). In all the cells examined, whether resistant or revertant, the relative amounts of dihydrofolate reductase parallels the number of gene copies for the enzyme.

One of the most intriguing questions posed by these results is why the cell adopts the strategy of gene amplification rather than increased gene transcription to gain drug resistance. There is a great deal of evidence to show that mRNAs in the cell occur in three abundance classes (see Bishop *et al. Nature* 250, 199; 1974; Lewin *Cell* 4, 77; 1975). The rare class contains very few copies (typically less than 20 per cell) of around 10^4 different mRNAs. The abundant class contains more than 10^4 copies of very few (up to 10) mRNAs and the moderately abundant class an intermediate number of copies (200–500) of about 500 different mRNAs (Hastie & Bishop *Cell* 9, 761; 1976). Most of these mRNAs are transcribed from genes present in only one copy per haploid genome (Hastie & Bishop *op. cit.*). This means that a cell can modulate gene expression over a 1,000-fold range without having to resort to gene amplification as a mechanism. There are certainly many cases where gene expression is altered so that a rare mRNA becomes more abundant and *vice versa*. Probably the best worked out example is the induction of mouse mammary tumour virus (MMTV) by steroid hormones (see *News & Views* 259, 628; 1976). In this case it seems very likely that the rate of transcription of the MMTV gene is increased several fold by the addition

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of glucocorticoids (Young *et al.* *J. Virol.* **21**, 39; 1977; Ringold *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 2879; 1977). These results show that a cell is capable of increasing the transcription rate of certain genes in response to certain external stimuli—why can't this be done when increased dihydrofolate reductase synthesis is required? The answer is unknown, but what is becoming clear is that the cellular genome need not be the constant entity it was once assumed to be. □

Ophiolites match oceanic crust

from Peter J. Smith

OPHIOLITE sequences are widely thought to be segments of ancient oceanic crust which have been upthrust, or otherwise emplaced, onto land. Indeed, taken together, the various pieces of evidence for this view make such a convincing case that few, if any, Earth scientists would care to oppose it. Yet the most convincing single piece of evidence has hitherto been absent, or at least available only in an unsatisfactory form. This is a direct one-to-one correlation of an ophiolite sequence with a section of modern oceanic crust. The problem is that ophiolites and oceanic crust are usually described in different ways; the former are defined largely in terms of petrology and field relations whereas the latter is defined in terms of seismic velocity structure.

One solution would be to obtain a complete petrological specification of typical *in situ* oceanic crust; but in spite of advances in drilling technology this is still not yet possible. So why not obtain the seismic velocity structure of an ophiolite sequence? This is quite possible; indeed Lort and Matthews (*Geophys. J.* **27**, 383; 1972) did it for the Troodos massif of Cyprus. The difficulty is, however, that ophiolite sequences are no longer under their original oceanic conditions and thus are no longer directly comparable with modern oceanic crust in seismic terms. Subaerial weathering, a decrease in hydrostatic confining pressure by erosion of upper levels, the drainage of water from pores and fractures following subaerial exposure, the introduction of new joints and fractures during the change in circumstances, and serpentinisation of ultramafics combine to reduce seismic velocities from their

original values by unknown amounts. Thus Lort and Matthews found Troodos velocities rather lower than they had hoped, thereby weakening (but not invalidating) the case for ophiolites as ocean floor.

But Salisbury and Christensen (*J. geophys. Res.* **83**, 805; 1978) have now overcome the problem of lack of comparability by measuring velocities, not of *in situ* ophiolites in the field, but of ophiolite samples under laboratory conditions which simulate the ophiolites' original circumstances as defined by geological context and stratigraphic level. Fresh samples were taken from all levels in an ophiolite sequence, and details of bedding, cumulate layering, tectonite banding, foliation and dyke and sill orientation were noted for the reconstruction of structure and stratigraphy. Both the P and S wave velocities of each sample were then measured over a range of confining pressures, and the velocities corresponding to the original *in situ* (as oceanic crust) pressure were obtained by interpolation.

The particular ophiolite sequence chosen by Salisbury and Christensen was the Blow-Me-Down massif of Newfoundland, part of the Bay of Islands ophiolite complex. The Bay of Islands complex in general is unusual in its degree of petrological completeness and its relatively simple structure; there is little evidence of post-emplacement metamorphism; most exposures are only slightly weathered, deeply weathered sections having been removed by late Pleistocene glaciation; there are sheeted dykes and cumulate layering to provide a structural frame of reference; and there are good and accessible exposures at all levels. Moreover, of four massifs within the Bay of Islands complex, Blow-Me-Down Mountain is the most perfect in these respects, although the topmost sedimentary layer is missing. This massif thus has an uppermost layer of pillow basalts overlying brecciated dykes which extend some way into the basalt layer above and into a sheeted dyke complex below. Next comes a layer of metagabbro overlying a layer of pyroxene gabbro which, in turn, overlies olivine gabbro and troctolites. Finally there is a basal layer of ultramafics.

This pile is thought (for example by Williams *et al.*, *Geol. Ass. Can. Spec. Pap.* **II**, 181; 1972) to be a remnant of Lower Ordovician oceanic crust and upper mantle. How, then, do the seismic velocities of the various layers as interpreted by Salisbury and Christensen compare with those of modern oceanic layers? The two sets of velocities are indistinguishable. Briefly, the ophiolite P wave velocity ranges from $\leq 5.70 \text{ km s}^{-1}$ at the top of the

basalt layer (crust) to 8.4 km s^{-1} in the ultramafic layer (mantle). The implication, therefore, is that oceanic layer 2 comprises pillow lavas and brecciated dykes, layer 3 consists of sheeted dykes underlain by gabbros of various types, and the Moho represents a relatively sharp transition from gabbro to dunite and peridotite (that is, a transition much sharper than those within the crust).

Of course, the fact that one particular ophiolite sequence is indistinguishable seismically from normal oceanic crust does not by itself prove the general validity of ophiolite-oceanic crust equivalence. But this example together with all previous evidence, the general similarity of ophiolite sequences and the overall uniformity of oceanic crust-mantle makes an already formidable case even more so. □



A hundred years ago

WHAT a surprise is in store for the children next Christmas if Mr. Edison's expectations are realised. Dolls that can say "papa" and "mamma," will be quite at a discount and will bear much the same relation to the doll of the future that the anthropoid ape does to the man of to-day, and the time will probably have come for some Darwinian toy-maker to write the history of doll development, if, indeed, he does not extend his researches to the whole world of toys. We are promised dolls that can speak, sing, cry, laugh; musical-boxes that will grind out the voice and words of the human singer; locomotives and every other species of "animal and mechanical toy," that will give out their natural and characteristic sounds.

But these are only some of the trifles to which Mr. Edison, in an interesting article in the current *North American Review*, tells us his miraculous invention will certainly or probably be put in the near future. And, indeed, a very little consideration will show that there is no end to the uses to which the principle of the phonograph may be applied; that it may be the means of actually realising some of the wildest dreams and speculations of the "frenzied" poet and preacher, and creating a revolution in human intercourse only to be paralleled by the invention of printing, or even of speech itself. Indeed, at first sight it may seem a step backwards, as it is likely to lead to the abolition, to some extent, of writing and printing, and the substitution of the human voice as the main means of intercourse at a distance.

From *Nature* **18**, 30 May, 116; 1878.

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articles

Radiocarbon timescale tested against magnetic and other dating methods

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A detailed comparison of conventional radiocarbon years with calendar years covering the past four centuries is given. Relatively large atmospheric ^{14}C changes are encountered over this time, and even very precise ^{14}C dating cannot entirely solve the problems of age calibration. By matching radiocarbon ages with ages derived from $^{230}\text{Th}/^{234}\text{U}$, thermoluminescence and magnetic dating, the ^{14}C timescale is shown to deviate by a maximum of 2,000 yr over the 9,000–32,000 yr BP interval.

A RADIOCARBON age is calculated by comparing the present-day measured ^{14}C activity with an atmospheric ^{14}C level which is assumed to have been constant in the past. This assumption, however, is only a first order approximation of reality, and radiocarbon ages often, therefore, deviate from calendar (solar) chronologies. Past atmospheric ^{14}C levels are determined by measuring the present-day ^{14}C activity of tree rings of known age. Palaeo- ^{14}C levels are then calculated by applying a correction for the ^{14}C decay since the time of formation of the wood. Several sequences of dendrochronologically dated trees exist, of which the longest continuous one is for bristlecone pine trees of the White Mountains. The bristlecone pine series has yielded palaeo-atmospheric ^{14}C levels back to ~7,500 yr BP (where BP is before AD 1950).

The tree-ring studies demonstrate convincingly the appreciable changes in past ^{14}C levels^{1–3}. A long-term change in ^{14}C levels causes radiocarbon ages to be 800 yr too young by 7,000 yr BP. This long-term increase in ^{14}C level between about 2,500 and 7,000 yr BP is well known, but the shorter term variations lasting a few centuries or less are more difficult to assess. Here two different aspects of the calibration problem are discussed: (1) the 'short-term' atmospheric ^{14}C variations during the past 450 yr; and (2) the possible long-term timescale changes beyond the time span covered by tree-ring research.

Calibration of the post-AD 1500 radiocarbon timescale

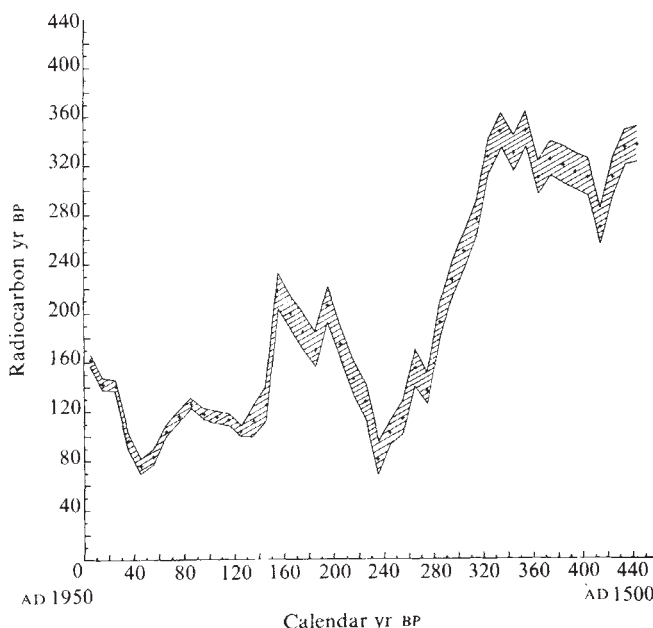
Wood from Douglas fir from Washington was used for the ^{14}C variations study. Although single year measurements are now being made to complete the record, the calibration curve reported here is mainly based on the measured ^{14}C activity of 10-yr (decadal) wood sections. For post-AD 1820 wood, single year measurements were averaged to give decadal means, resulting in smaller standard deviations for

this time interval (Fig. 1). The precision of each ^{14}C measurement was 2‰ or better, which is equivalent to an age error of 16 yr or less. The specific details of the experimental technique will be reported elsewhere (in preparation).

For most of the samples reported here the de Vries type of sample treatment was used. This treatment does not, however, remove all components added to the wood after the year of formation^{4,5}. Extensive experiments with Douglas fir wood show that the feedback of the natural variations, with the de Vries treatment, results in maximum errors of 0.3‰ in ^{14}C , or 2.4 radiocarbon yr. For twentieth-century wood the influence of nuclear bomb ^{14}C is much more pronounced, and pure α cellulose fractions were used for the post-AD 1910 data points in Fig. 1.

An important aspect of ^{14}C studies are the climatic implications. A detailed comparison between ^{14}C and climatic record will be made elsewhere, together with carbon

Fig. 1 The relationship between conventional radiocarbon ages²² (5,568 yr half life) and tree-ring calibrated calendar years. (Different calendar years often have the same radiocarbon age). The width of the curve is twice the counting error in the measurements. The total error in the measurement process is only a few 0.1‰, larger than the counting error. 0 yr BP is AD 1950.



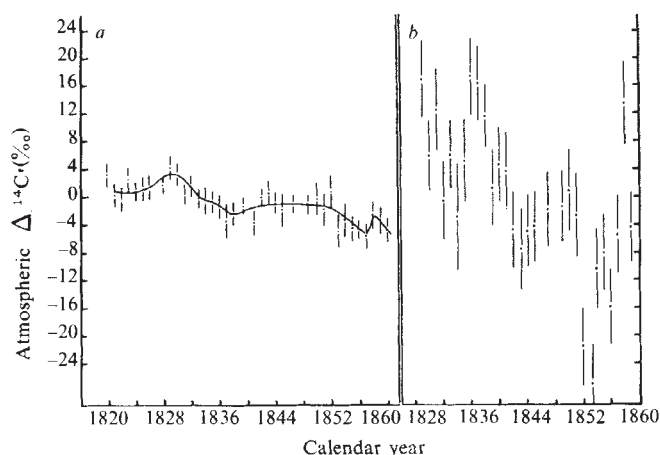


Fig. 2 A comparison of the atmospheric ^{14}C variability obtained from two series of measurements, one for Douglas fir from Washington (a) 48°N and one for oak⁷ from the Forest of Dean (b) 52°N .

reservoir modelling of the ^{14}C variations. The reduction in twentieth century atmospheric ^{14}C levels by industrial CO_2 release will also be discussed elsewhere. This anthropogenic lowering of ^{14}C level (Suess effect) results in an increase in radiocarbon ages for the twentieth century (Fig. 1).

The width of the calibration curve in Fig. 1 is twice the standard deviation in the ^{14}C activity measured for single decade samples. The curve shows that a lifetime in radiocarbon years would have many surprises: the years can be stretched or compressed, or even change sign. Instead of ageing by 100 calendar years in the eighteenth century, one would be 130 radiocarbon years younger near the end of the century. In the seventeenth century, however, the ageing process accelerates to about 260 radiocarbon yr. Clearly, the short-term calibration problems are of major concern in chronological studies where age separation of about a century is needed.

Even when a radiocarbon age is determined with a precision of ± 16 yr, or better, there is only one interval of a few decades near AD 1650 that potentially yields only one calendar date for one radiocarbon age. In all other instances either multiple calendar dates, or a much broader continuous spectrum of calendar ages, are derived from a single radiocarbon date.

Not all time intervals have the fairly large ^{14}C variability found for the past four centuries. Pearson *et al.*⁵ have shown ^{14}C variability around the main trend to be only $\pm 6\%$ (± 50 yr) in a European oak series between 3,600 and 4,600 radiocarbon yr BP. Here the variability of ± 50 yr over $\sim 1,000$ yr interval is much less than the variability of $\sim \pm 120$ yr encountered in the Douglas fir study over the past 450 yr. The oak study demonstrates the existence of time intervals where detailed age calibration will yield more positive results than given here for the past 450 yr.

Previous reports by Suess on bristlecone pine wood³ show a somewhat larger ^{14}C variability than found in the European oak⁶. Similarly, although our study shows in itself large changes on a century scale, the work on single-year tree rings shows much less variability in annual ^{14}C changes than reported by Baxter and Farmer for an English oak from the forest of Dean⁷. The results obtained for the forest of Dean oak were thought to prove the existence of an 11-yr-solar cycle with a ^{14}C amplitude of several per cent change in ^{14}C . These data conflict with a twentieth-century tree ring series measured at the University of Arizona⁸, and also are contradicted by the small year to year changes in the Pacific Northwest Douglas fir (Fig. 2). In fact, a more

extensive series of single-year Douglas fir measurements, each with 2‰ precision, do not show statistically significant 11-yr periodicity between AD 1820 and 1950 (M.S., in preparation).

The standard experience with analytical work is that experimental problems introduce larger variability. More interlaboratory calibration checks of high precision are needed to solve some of the problems mentioned. One should remember, however, that not all ^{14}C variability on a century scale can be attributed to experimental problems. For instance, in the Douglas fir study the difference in atmospheric ^{14}C level between the beginning and end of the eighteenth century is equal to 12 times the standard deviation of single measurements.

Radiocarbon timescale changes between 9,000 and 32,000 yr BP

Because the 2,000–7,000 yr long-term change in atmospheric ^{14}C level results in a radiocarbon age anomaly of 800 yr for 7,000-yr-old samples, the possibility of a much larger age discrepancy further back in time exists. It is shown here that such age anomalies are most likely restricted to less than 2,000 yr over a 32,000-yr-interval.

Some evidence comes from the $^{230}\text{Th}/^{234}\text{U}$ ages of Searles Lake sediment. This Californian lake has been desiccated in modern times, but it has experienced several pluvial intervals in the past. A large number of published ^{14}C dates give the absolute chronology of the sediments^{9–10}. Additional ^{14}C analysis of organic materials of the so-called Lower Salt stratum gave a very precise ^{14}C record¹¹. This was used by Peng *et al.*¹² for a comparison with their ^{230}Th chronology of Lower Salt deposits.

The ^{230}Th ages are based on absolute decay rates, and are fully independent of atmospheric ^{14}C changes. Agreement between both dating methods would therefore indicate the lack of appreciable anomalies in ^{14}C dates due to atmospheric ^{14}C changes.

A comparison of the ^{14}C and ^{230}Th ages is given in Fig. 3 for the Lower Salt sediments (the 22,000–32,000-yr series). The thorium ages are for salt layers bedded between organic muds. The ^{14}C ages in Fig. 3 are obtained by taking the mid-point of the ^{14}C age of the top of the organic layer below the salt layer; and the ^{14}C age of the bottom portion of the organic layer above the salt. The standard errors in the ^{14}C determinations are ~ 400 yr. The standard errors in the Th measurements depend on the number of samples analysed per salt layer and range from 800 to 1,500 yr. All data points (○) in Fig. 3 are within 2 standard deviations from the ideal one-to-one relationship. We conclude that this type of agreement can only be obtained if both methods give ages close to the actual age of deposition. ^{14}C age anomalies exceeding 2,000 yr seem unlikely over the 22,000–32,000 yr BP interval.

Further evidence on the reliability of ^{14}C dating can be derived from Berry's thermoluminescence studies of Hawaiian basalts¹³. Four tholeiitic basalt flows from Mauna Loa and Kilauea are part of these studies. The ^{14}C ages were derived from organic materials associated with these flows. The specific thermoluminescence correlates linearly with the ^{14}C ages ranging from $\sim 3,000$ yr to 17,000 yr. The crossed data points in Fig. 3 near 10,100 and 17,400 radiocarbon yr were derived from this study.

The cyclicity in the pattern of secular variation of the geomagnetic field provides additional information on long-term changes in atmospheric ^{14}C content. A classic example of these variations is found in sediment of Lake Windermere¹⁴. There are clear patterns of east to west cyclic shifts in declination, caused by changes in the non-dipole component of the Earth geomagnetic field. Although the

position of the east-west maxima cannot always be precisely determined, the magnetic declination pattern shows an amazingly stable periodicity over the past 8,000 tree-ring calibrated years (Fig. 4). The older ^{14}C date beyond tree-ring calibration (maximum 8) also falls on the line if an age correction of 800 yr is applied at about 11,000 yr BP. This implies a 10% higher atmospheric ^{14}C level near 11,000 calendar yr BP. However, the last maximum is not precisely determined magnetically, and more evidence is needed to support the above conclusion. Varve studies of Lake of the Clouds, however, do support the concept of a similar higher ^{14}C level for early Holocene time¹⁵.

The main geomagnetic dipole does not seem to be the source of the secular oscillations¹⁶. The oscillations are caused by the non-dipole Earth magnetic field, of which the intensity is only a fraction of the main dipole intensity. Thus, the oscillations of the non-dipole component itself cannot materially change atmospheric ^{14}C production. The ^{14}C dates therefore are independent of the registered non-dipole geomagnetic oscillations.

The contours of the non-dipole field form several closed loops around centres of maximum strength, and can be explained by a number of radially placed dipoles in the Earth's outer core. The magnetic oscillations of these radial dipoles need not have identical periodicities. Detailed ^{14}C control is often lacking in geomagnetic profile studies, and a similar stable periodicity as found for Lake Windermere has been found only in Black Sea sediment¹⁷. Here Creer used ^{14}C dating to demonstrate a constant periodicity of magnetic inclination similar to that of Lake Windermere. Our assumption of constant periodicity is a weak point in the ^{14}C against magnetic age calibration, but it seems to be a very reasonable and straightforward concept. The excellent agreement between the magnetic and ^{230}Th date near 24,000 yr BP (Fig. 3) supports the soundness of the assumption of constant periodicity of magnetic variations.

At a depth of 55 cm in the Black Sea core the first detectable minimum in geomagnetic inclination is encountered. The approximate ^{14}C age at this depth is 6,040 yr (based on interpolation between two dates of 7,000

Fig. 3 A comparison of ^{14}C ages with ages determined through other geochronological methods. These include $^{230}\text{Th}/^{234}\text{U}$ dates on lake sediments ($\circ$), thermoluminescence dates of basalt ($\times$) and magnetic dates ($\bullet$). Vertical and horizontal bars denote one standard deviation. The dashed line indicates the ideal one-to-one relationship.

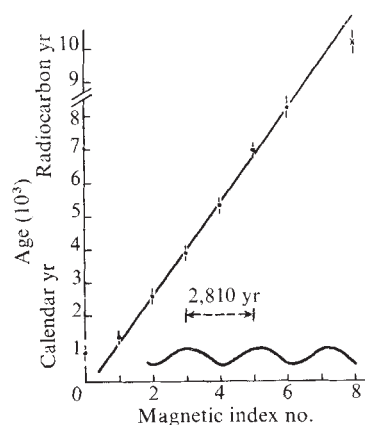
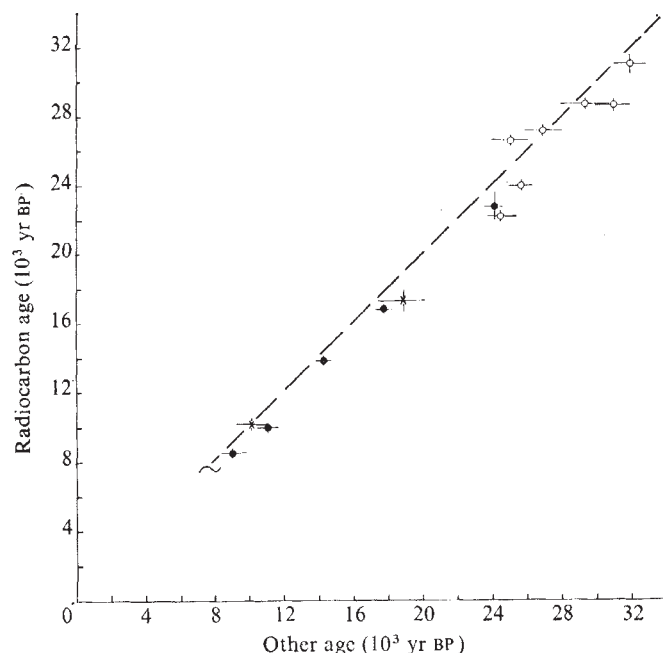


Fig. 4 Geomagnetic declination oscillation index number of Lake Windermere sediment plotted against tree-ring corrected ^{14}C dates. The index number was obtained by consecutive numbering of the geomagnetic extrema in declination. Tree-ring age calibration of the ^{14}C date near 10,000 yr ($\times$) is not possible. The sinusoidal curve is a visual aid only.

and 3,090 yr BP (ref. 18)). The actual magnetic periodicity in the Black Sea region is not known because measurements covering the past 6,000 yr are lacking. Hence the assumption of equal periodicity as encountered at Lake Windermere.

The tree-ring calibrated age for a radiocarbon age of 6,040 yr BP is about 6,600 calendar yr. Magnetic ages (M) down the core are here calculated according to $M = 6,600 + 2,810S$, where S equals the number of magnetic cycles below the first inclination minimum at 55 cm depth in the core. There are four ^{14}C ages available for this core beyond the tree-ring chronology: $8,600 \pm 150$ yr BP at a depth of 120 cm; $13,850 \pm 210$ yr BP at a depth of 330 cm; $16,900 \pm 270$ yr BP at a depth of 580 cm; and $22,830 \pm 800$ yr BP at a depth of 1,120 cm (ref. 18). The number of magnetic cycles, S , below the first inclination minimum in the core are, respectively, 0.82, 2.7, 4.0, and 6.2 at these depths¹⁷. Thus magnetic ages of, respectively, 8,900, 14,200, 17,800, and 24,100 yr are obtained.

The data points ($\bullet$) in Fig. 3 are based on the above comparison of ^{14}C and magnetic ages. The error bar in the magnetic age is calculated by estimating the error in the determination of the position of the magnetic inclination to be $\pm 0.2 S$ units.

The ^{14}C ages, as plotted in Fig. 3, deviate at a maximum 2,000 yr from the ideal one-to-one relationship. Due to the fairly wide spacing of the data points, it is possible to draw a curve with some oscillations at selected ages. However, proof for such oscillations is lacking. The data given here should conservatively be interpreted as evidence for limited ^{14}C timescale variability between 9,000 and 32,000 yr BP.

Nearly all Fig. 3 points have ^{14}C ages slightly too young; these were calculated with the conventional 5,568 yr half life. Using the more precise 5,730 yr value for the half life increases the ^{14}C ages by 3%. Such a correction improves the agreement between ^{14}C ages and ages derived from the other methods, and systematically younger ^{14}C ages are no longer a problem. The half life correction reduces the average radiocarbon age dispersion (Fig. 3) to only 700 yr.

Varve studies of Lake of the Clouds sediment have shown that somewhat higher atmospheric ^{14}C levels were encountered in the early Holocene¹⁵. An increase in this anomaly further back in time seems unlikely in view of the good age agreement cited above. Evidently, the changes in ^{14}C distribution between atmosphere and oceans are relatively small for glacial-interglacial climatic changes.

Different processes, working in the opposite direction, seem to influence atmospheric ^{14}C level during glacial

episodes. A reduction in ^{14}C atmosphere-ocean exchange rate may be caused by lower sealevel and increased ice cover. Downward advection of ^{14}C into the deep ocean may be less because of a reduced rate of bottom water formation in the Atlantic^{19,20}. A reduced rate of bottom water formation also reduces world-wide rate of oceanic upwelling, and brings less ^{14}C deficient water to the surface. All the above processes would point to a higher atmospheric ^{14}C level. However, oceanic thermal gradients are less during glacials, and downward eddy diffusive transport of ^{14}C over the oceanic thermocline increases. Such transport also increases when vertical upward advection velocities are reduced. Whatever processes are occurring, their variability during glacial and interglacial times evidently has not resulted in a large change in atmospheric ^{14}C level.

Distortions in ^{14}C timescale can also be introduced by the Earth's geomagnetic field reversals. Age anomalies up to a few thousand years can be expected for 'events' lasting a few thousand years²¹, and are not entirely excluded by the data presented here. It should also be noted that thermoluminescence and ^{14}C dates of the Lake Mungo geomagnetic polarity excursion near 30,000 radiocarbon yr BP agree with each other within the 4,300 yr thermoluminescence age error²³.

It has been standard practice in geochronology to compare other dating methods with ^{14}C dating mainly to prove the reliability of these other methods. Here, the best examples of these other methods (^{230}Th , thermoluminescence and magnetic dating) are used to check on the reliability of

^{14}C dating. The information thus obtained supports the concept of a reliable ^{14}C timescale back to 32,000 yr BP, within a maximum error of about 2,000 yr.

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An association between actin and the major histocompatibility antigen H-2

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P815 cells spontaneously shed material (exfoliate) in which actin is the major protein. The exfoliate also contains H-2. The association between actin and H-2 is stable even in the presence of detergent, indicating that protein-protein interactions exist between the actin and H-2. The exfoliate probably consists of highly purified microvilli.

THE possibility of transmembrane associations between external and internal proteins across the plasma membrane of the cell has often been considered¹⁻⁴. The concept has several appealing features. In principle, it provides a mechanism by which certain types of extracellular signals can be transmitted to the interior of the cell. For example, mitogens such as lectins can exert their effects on DNA synthesis and cell division without actually entering the cell^{5,6}. However, if the receptors are capable of transmembrane associations with intracellular components, the extracellular event can be transferred to the interior of the cell by a variety of possible perturbations of the transmembrane association.

The putative transmembrane associations which have received most attention are those involving the cytoskeletal elements of the cell, because of their apparent involvement in the redistribution of surface receptors during the capping phenomenon⁷. It is envisaged that capping occurs because surface receptors become attached to cytoskeletal elements such as microfilaments and microtubules, which are intrinsically capable of redistribution within the cell. Many studies have

provided evidence of varying degrees of directness to support this hypothesis. The early evidence was obtained from observations on the effects of drugs such as cytochalasin b and colchicine on capping, which suggested that microfilaments and microtubules were involved (reviewed in ref. 2). More recently, it has been found that cells which contain capped receptors have a similar redistribution of cytoskeletal elements such as actin and tubulin⁸⁻¹⁰. This parallel rearrangement of surface receptors and cytoskeletal proteins has also been observed during the more subtle rearrangements which occur during patching¹¹.

Transmembrane associations between surface proteins and cytoskeletal elements are also interesting because they suggest a device for attaching these elements to membranes. It is known that microfilaments, in particular, make contact with the plasma membranes of cells¹²⁻¹⁶, and these associations are probably important in phenomena such as the formation of microvilli and the motility of cells¹⁷⁻²⁰. However, the molecules involved in the attachment of microfilaments to membranes have not been identified. Evidence that α -actinin is involved in the attachment is available, but inconclusive^{21,22}. An alternative, which does not necessarily exclude a role for α -actinin, is that a transmembrane protein is ultimately responsible for the attachment.

In spite of the considerable interest in the putative transmembrane associations between surface receptors and cytoskeletal elements, direct evidence for an association between these components is lacking. We report here evidence that in at least one system an exoprotein, the major histocompatibility antigen H-2, forms a stable association with the microfilament protein actin. This association exists in the intact cell and does

not depend on the presence of the plasma membrane. As H-2 is a transmembrane protein^{23,24}, it is possible that the association is direct. The available evidence suggests that the complexes exist in microprojections of the cell, resembling microvilli.

Exfoliation, the shedding of specific membrane components

The P815 cell line, which has been identified as a murine mastocytoma²⁵, was found to shed large amounts of plasma membrane-derived material. The process, which is referred to as exfoliation, is easily detected when freshly collected cells are incubated at 37 °C. The type of medium used is not important, but the exfoliation does not occur at a detectable rate if the cells are cooled. It is complete within 90 min (Fig. 2). The striking feature of the exfoliation is the presence of a large amount of a single protein in the shed material (Fig. 1A) which has been identified as actin by one- and two-dimensional gel electrophoresis, binding to myosin, mapping of tryptic peptides and amino acid analysis. The actin seems to be in the filamentous form, as it can be pelleted in the presence of detergent (see below). On average, the shed actin accounts for about 5% of the total cell actin. However, most of this apparently originates from the membrane-associated actin (~10% of total cell actin), as there is a close reciprocal relationship between the amount of actin occurring in the exfoliate and that lost from membranes in cells during the exfoliation. Thus, up to half the membrane-associated actin can be lost during the shedding.

The exfoliation has two other important features; first, it is selective, that is, only some membrane-associated components are shed, and second, there seems to be an enrichment of these components in the exfoliate. The selectivity is apparent from the two-dimensional maps of the glycoproteins in membranes and exfoliate (Fig. 1B). Thus, only a small proportion of both proteins and glycoproteins from the membrane preparation are found in the exfoliate. Selectivity was also evident when the shedding of surface antigens was compared (Fig. 2). Thus, when almost half the surface H-2 was shed during the exfoliation, the viral antigen P30, which is also present in significant amounts on the surfaces of these cells, was only shed very slowly and to a very much smaller extent. The P30 shedding is probably an index of the small amount of cell lysis (<5%) which occurs during the exfoliation.

Perhaps the most striking feature of the shedding is the apparent enrichment of the exfoliate for certain plasma membrane-associated components. This conclusion relies on the observation that the shedding does not involve a general release of plasma membrane. Estimates of the amount of gross release of plasma membrane were obtained from studies on the binding of ¹²⁵I-concanavalin A. It was found that after shedding, the cells had lost <10% of their concanavalin A receptors, suggesting that the total proportion of cell surface lost was of this order. In contrast, some cell surface antigens, notably the major histocompatibility antigen H-2, were apparently depleted by as much as 50%, after the shedding (Fig. 2a). Estimates of this order were obtained when shed and unshed cells were compared, when membranes from shed and unshed cells were compared, and when the amount of exfoliated H-2 was compared with that remaining on the cells after shedding. All these estimates showed that up to half the surface H-2 could be shed during the exfoliation. As mentioned above, about half the membrane-associated actin was apparently also shed during the exfoliation. As the available estimate for total cell surface release is of the order of 10%, the implication is that the actin and H-2 are enriched in the exfoliated membranes. Such an enrichment for particular membrane-associated components could arise if the shedding resulted from the release of membranous regions which were already enriched for actin and H-2. This possibility was supported by the observations that the shedding of actin and H-2 is very closely coordinated (Fig. 2b), which would be expected if they arose from the same process, and that all the

actin and H-2 band at a density of about 1.156 when subjected to sucrose gradient centrifugation (Fig. 2c).

Thus, the above data suggest that exfoliation results from the exocytosis of specific regions of the plasma membrane which are particularly rich in actin and H-2.

Association of actin and H-2 in exfoliate

As H-2 molecules are generally thought to be capable of relatively free movement in the membrane^{26,27}, their apparent localisation in particular areas of the membrane requires a restraining mechanism in these areas. The obvious possibility is that the actin serves this function as a consequence of an association, direct or otherwise, with the H-2. To test this, the membrane in the exfoliate was dissolved with detergent and the actin pelleted by centrifugation at high speed (Fig. 4a). The

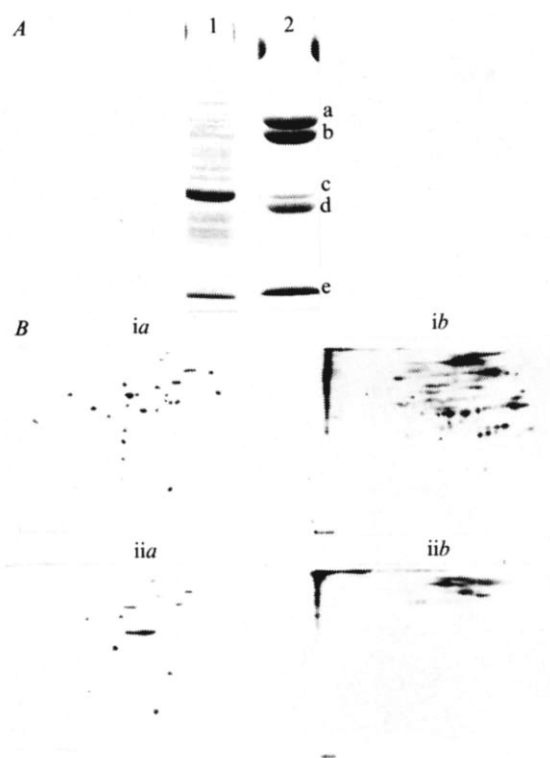


Fig. 1 Proteins and glycoproteins in P815 exfoliate. P815 cells were grown to a density of 5×10^5 cells per ml in RPMI 1640 medium (Flow) with 10% calf serum, 4 mM glutamine, 100 units per ml penicillin and 100 units per ml streptomycin. Cells were collected by centrifugation at 400g for 10 min at 4 °C, washed twice with phosphate buffered saline (PBS) and suspended at 10^7 cells per ml in PBS. The cells were incubated at 37 °C for 90 min and pelleted at 1,000g for 10 min. The supernatant was layered on to a cushion of 45% (w/v) sucrose in PBS and centrifuged at 100,000g for 60 min at 4 °C. The material at the top of the sucrose cushion (exfoliate) was gathered, diluted and collected by pelleting at 100,000g for 60 min. Membranes were prepared by hypotonic lysis, homogenisation and sucrose gradient centrifugation³¹. Two-dimensional gel electrophoresis was carried out by the method of O'Farrell³², sodium dodecyl sulphate (SDS) gel electrophoresis by the method of Laemmli³³ and glycoprotein staining with ¹²⁵I-concanavalin A by the method of Robinson *et al.*³⁴. Protein staining on gels was with Coomassie blue. **A**, One-dimensional SDS gel electrophoresis of exfoliate. 1, Purified exfoliate from 4×10^7 cells; 2, marker protein mixture containing a, human transferrin; b, bovine serum albumin; c, rabbit muscle actin; d, chicken ovalbumin; e, soybean trypsin inhibitor. **B**, Two-dimensional gel electrophoresis of exfoliated proteins and glycoproteins. Samples of membranes (2×10^6 cell equivalents) and exfoliate (4×10^7 cell equivalents) were prepared as described above and subjected to two-dimensional mapping. Gels were stained for both proteins and glycoproteins using the same gel for both stains. Drawings of the protein patterns are shown. Upper pattern: a, membrane, protein stain; b, Membrane, glycoprotein stain. Lower pattern: a, exfoliate, protein stain; b, exfoliate, glycoprotein stain.

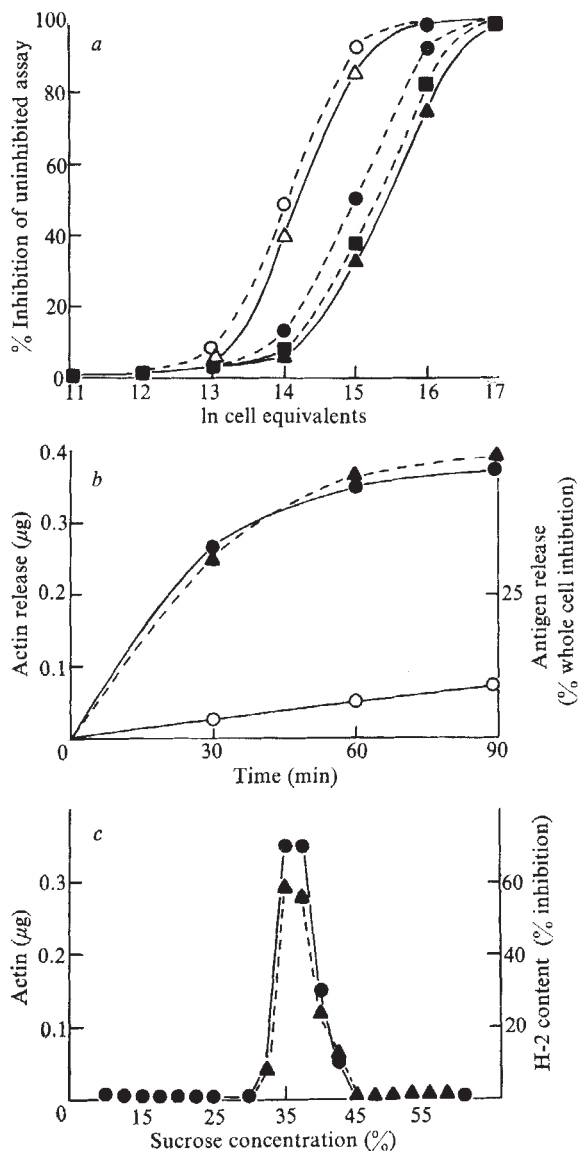


Fig. 2 Release of actin and H-2 during exfoliation. *a*, Release of H-2 from cells during exfoliation. Cells were prepared for exfoliation as described in the legend to Fig. 1. One sample of cells was incubated at 37 °C for 90 min and a control sample was kept on ice. No exfoliation occurs in the latter sample. The exfoliated material was collected as described in the legend to Fig. 1. Aliquots of the two samples of cells were used to prepare membranes. The H-2 content in all samples was estimated by measuring their absorbing capacity for specific anti-H-2^d serum (BIOBr anti-BIOD2, Searle). The anti-H-2^d serum was measured by the two-step ⁵¹Cr-release cytotoxicity assay³⁵ using P815 cells as targets and rabbit serum as the source of complement. △, Cells at 4 °C; ▲, cells at 37 °C; ○, membranes from 4 °C cells; ●, membranes from 37 °C cells; ■, exfoliate. *b*, Coordinate release of actin and H-2. Freshly collected cells were washed and suspended at 10⁷ cells per ml in PBS and exfoliated at 37 °C. Aliquots of the incubation medium were removed at intervals and the exfoliate collected as described in the legend to Fig. 1. The H-2 in each sample of exfoliate was measured by absorption of the anti-H-2^d serum and P30 was measured by the same assay using rabbit anti-P30 antibody. P815 cells were used as targets in both assays, and both antibody concentrations gave a 50% lysis of cells in the unabsorbed assay. Actin in the exfoliate was quantitated by densitometry on stained SDS gels of each sample. 1 × 10⁷ cell equivalents were used for each estimate. ▲, actin; ●, H-2; ○, P30. *c*, Sedimentation of actin and H-2 on sucrose gradients. 4 × 10⁷ cells were exfoliated as described in the legend to Fig. 1 and the whole exfoliation medium (minus cells) was applied to the top of a 15–60% sucrose gradient in PBS (total volume 10 ml) and centrifuged at 100,000g at 4 °C for 16 h. Samples were collected from the gradient, concentrated by dilution and pelleting, and analysed for H-2 and actin as described above. ▲, H-2; ●, actin.

actin pellet was assayed and found to contain all the H-2 which had been in the untreated exfoliate. This is consistent with the possibility that the actin and H-2 are actually associated with one another even in the absence of the plasma membrane.

To obtain more rigorous evidence for this association between actin and H-2, an approach called the myosin affinity technique was applied to the detergent-stripped exfoliate. The strategy of this technique is to make use of the strong and specific affinity of myosin filaments for actin. Thus, if a sample contains actin in association with some other protein, it should be possible to bind the latter to myosin filaments through the actin. Furthermore, when binding of a protein to myosin filaments is detected, the involvement of actin in this binding can be confirmed by presaturating the myosin filaments with actin which ought to inhibit the binding of the putative actin-associated protein. The specificity of synthetic myosin filaments for actin is shown in Fig. 3*a*. In studies with lymphocyte lysates, it was found that H-2 itself does not have an affinity for the myosin filaments. Therefore, in both the general and particular cases the myosin filaments seem to be very specific for actin.

Application of the myosin filaments to the detergent-treated exfoliate shows that the actin does bind to the filaments (Fig. 3*b*). From studies using radioactively labelled exfoliate, it was found that the binding could be inhibited by presaturating the myosin filaments with rabbit muscle F actin. Serological analyses of the myosin filaments after mixing with the detergent-treated exfoliate show that virtually all the H-2 can be bound to the myosin filaments, and that the binding of the H-2 can be inhibited by presaturating the myosin filaments with actin (Fig. 4*b*). Thus, H-2 in the exfoliate satisfies the criteria for an actin-associated protein.

Is the exfoliate composed of detached microvilli?

Some of the studies described above indicated that the exfoliate contains a disproportionate amount of the membrane actin and H-2, suggesting that the complexes between these components were localised into particular areas of the cell membrane which were easily shed. Thus, the origin of the exfoliate is a relevant question. Several observations exclude cell lysis as the source of exfoliate. First, numerous studies have not detected even 5% cell lysis during the shedding. Second, when cells are actually lysed and the products isolated exactly as described for the exfoliate, the pattern of proteins and glycoproteins is even more complex than that obtained from the membrane preparation shown in Fig. 1*B*. Thus, general cell lysis is inconsistent with the selectivity of the exfoliation. Finally, it is difficult to reconcile the lysis of a few cells with the release of such a large proportion of the membrane actin and H-2.

Studies using scanning electron microscopy have shown that the P815 cell is covered with large numbers of microprojections ranging from 0.2 to 4 μm (ref. 28). This was confirmed for the cells used in the present study using Nomarski interference microscopy, which showed that the microprojections could be as long as 15 μm, with an approximate diameter of 0.1 μm. As these microprojections have been called microvilli the latter term will be used. When cells in culture are examined, large numbers of the microvilli are found to cover the cell completely (Fig. 5*a*). However, after the exfoliation there is a distinct decrease in the number of microvilli and the cells seem to be smoother. Also, it is found that many of the microvilli are free in the exfoliating medium, indicating that they have been detached from the cell during the incubation. The ease with which the microvilli are detached from the cell body is apparent when the coverslips used to attach the cells for examination are gently washed with medium. Many cells are detached, but they leave behind dense imprints of microvilli. Therefore, although formal proof is lacking, the appearance of free microvilli during the exfoliation, the ease of detachment of the microvilli from the cell body, the absence of other detectable material, the absence of general cell lysis and the presence of a large amount

of filamentous actin in the exfoliate, all suggest that the exfoliate consists of detached microvilli.

Discussion

The main conclusion drawn from the above studies is that the major histocompatibility antigen H-2 can form a complex with the membrane-associated actin of the P815 cell. The complex was isolated from material which was shed spontaneously by this cell line, and the evidence suggests that the shed material consists mainly of detached microvilli. Shedding of plasma membrane through exocytosis of microprojections has been observed in certain other systems, and it is possible that it serves as a general mechanism for the turnover of normal membrane and for ligand-induced shedding of various membrane antigens²⁹. However, the process apparently occurs at an enhanced rate in the P815 cell, and the reason for this is obscure.

The feature of the H-2-actin complex in the exfoliate is the fact that it does not rely on the presence of the plasma membrane lipid. Therefore, the association seems to depend solely on protein-protein interactions. The possibility of such an association between exoproteins and a cytoskeletal protein has been suggested before¹⁻², but direct evidence has been lacking. However, it must be emphasised that the available evidence does not distinguish between a direct attachment of the H-2 to the actin filaments and one involving one or more intermediate elements.

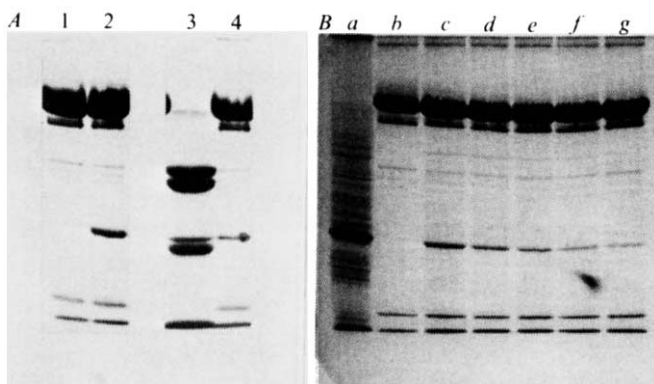


Fig. 3 The myosin affinity technique for actin and actin-associated proteins. Myosin filaments were prepared by diluting purified rabbit skeletal muscle myosin (stored at -20°C in 0.6 M NaCl and 50% glycerol) into 10 mM Tris-HCl, pH 7.5, at a final concentration of 1 mg per ml. Filaments, which form within 15 min at 4°C , were centrifuged, washed in the Tris buffer and finally suspended at 1 mg per ml in Tris buffer with 1% Nonidet P40. For binding experiments 50 μg of myosin filaments were pelleted and mixed with test samples in 100 μl of Tris/NP40 buffer for 1 h at 4°C . The myosin filaments were pelleted at 1,000g for 5 min, washed 3 times with Tris/NP40 buffer, analysed for radioactivity and/or subjected to SDS gel electrophoresis according to the method of Laemmli³³. The resolving gel consisted of 5% acrylamide layered on 7.5% acrylamide before polymerisation. This prevents distortion of bands by the large amount of myosin present in samples. Gels were stained with Coomassie blue for proteins. For presaturation of myosin filaments with actin, 200 μg was mixed with 1 mg of pure rabbit skeletal muscle F actin in 500 μl of Tris buffer and incubated at 4°C for 4 h. The actomyosin was washed and used as described above for myosin filaments. **A**, Specificity of myosin filaments for actin. The mixture contained human transferrin (50 μg), bovine serum albumin (50 μg), rabbit skeletal muscle actin (10 μg), chicken ovalbumin (50 μg) and soybean trypsin inhibitor (50 μg). The samples on the gel are 1, myosin filaments; 2, presaturated myosin filaments; 3, synthetic mixture of proteins; 4, myosin filaments treated with the synthetic mixture of proteins. Staining was for proteins using Coomassie blue. **B**, Application of myosin affinity technique to detergent-solubilised exfoliate. Purified exfoliate was prepared as described in Fig. 1, and 1×10^8 cell equivalents were made up in 200 μl of 10 mM Tris, pH 7.5, with 1% Nonidet P40. The sample was spun at 10,000g for 6 min to remove any dense material, and serial dilutions prepared, each in 100 μl of the Tris/NP40 buffer. 200 μg of myosin filaments were added and the samples stood at 4°C for 1 h. The myosin filaments were washed 3 times with Tris/NP40 buffer and subjected to SDS gel electrophoresis as described above. Gels were stained with Coomassie blue as described above. **a**, Exfoliate; **b**, myosin; **c-g**, myosin and serial dilutions of exfoliate.

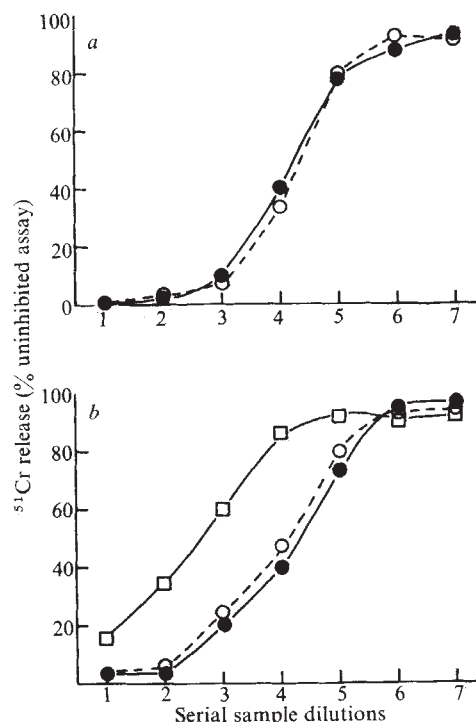


Fig. 4 Association between actin and H-2 in detergent treated exfoliate. **a**, Sedimentation of actin and H-2 in detergent-treated exfoliate. Exfoliate from 10^8 cells was suspended in PBS with 1% NP40 and centrifuged for 1 h at 100,000g. The pellet (which contains all the actin) was resuspended in PBS and analysed for H-2 by absorption of anti-H2^a serum. An equivalent sample, not treated with detergent, was centrifuged in parallel for comparison. ●, Detergent-treated sample; ○, untreated sample. **b**, Application of the myosin affinity technique to detergent-treated exfoliate. Exfoliate from 1×10^7 cells was made up in 50 μl of 10 mM Tris-HCl, pH 7.5, with 1% NP40 and centrifuged at 10,000g for 6 min to remove any dense material. The supernatant was mixed with 200 μg of myosin filaments as described in the legend to Fig. 3. After 6 h at 4°C to ensure complete binding, the myosin filaments were washed with 10 mM Tris-HCl, pH 7.5, and dissolved in 50 μl of PBS. In a parallel experiment, the same procedure was used except that the myosin filaments were presaturated with rabbit skeletal muscle F actin by mixing 200 μg of myosin filaments with 1 mg of F actin, incubating for 1 h at 4°C and washing with this buffer. The exfoliate-treated myosin and actomyosin were assayed for H-2 by absorption of anti-H2^a serum. A sample of the untreated exfoliate was included for comparison. Myosin filaments show no absorption in the cytotoxicity assay. ●, Exfoliate; ○, myosin and exfoliate; □, actomyosin and exfoliate.

More detailed analyses of the detergent-stripped exfoliate using cross-linking and other techniques will be necessary to distinguish between these possibilities. Nevertheless, whether the association between actin and H-2 is direct or not, the existence of a transmembrane association of some sort seems inescapable. The main argument in favour of a direct association is the fact that histocompatibility antigens are themselves transmembrane proteins^{23,24} and can, in principle, bind directly to the intracellular actin. It should be noted in this connection that the available evidence does indicate that the H-2-actin association exists in the cell before the exfoliation occurs. Thus, the close parallel in the rates and extents of shedding of H-2 and actin and the absence of actin-free H-2 in the exfoliate argue against the possibility that they come together after the shedding. Furthermore, in unpublished studies in which the myosin affinity technique was applied to detergent-lysates of whole cells and membranes, most of the H-2 was found to be actin-associated. Therefore, it must be presumed that the putative transmembrane association mentioned above must exist in the intact cell. Consequently, studies on the isolated H-2-actin complex are likely to be relevant to the arrangement of these components in the intact cell.

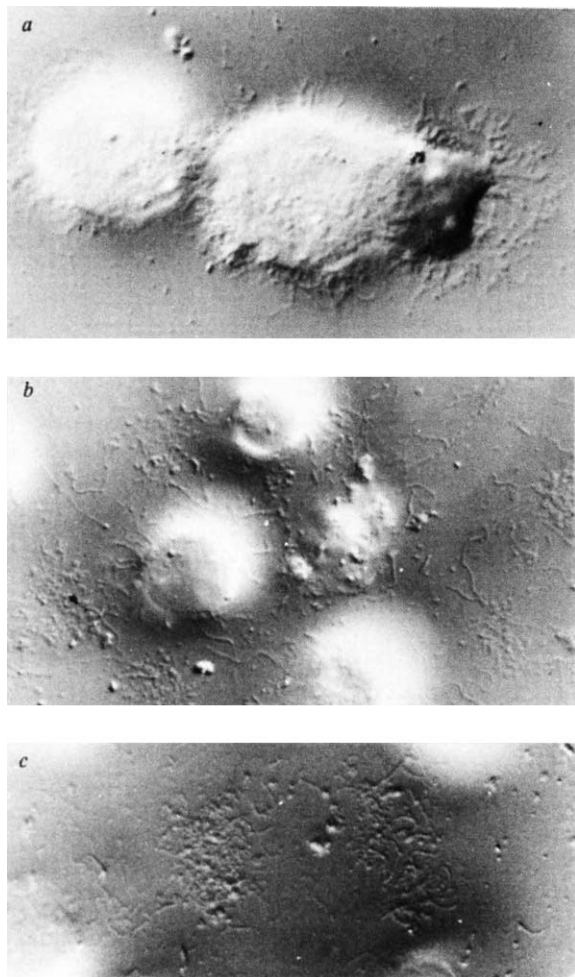


Fig. 5 Microprojections on P815 cells. Live cells were attached to polylysine-coated glass coverslips by placing a droplet of cell suspension on the coverslips and allowing it to settle for 15 min at room temperature. The coverslips were rinsed thoroughly with PBS and mounted, and the cells examined by light microscopy using Nomarski interference optics. *a*, Cells absorbed directly out of a log phase culture; *b*, exfoliating cells; *c*, microvillar tracks from exfoliating cells. Scale: *a*, 1 cm - 4 μm; *b*, *c*, 1 cm - 6 μm.

The H-2-actin complex detected here is somewhat unusual in the sense that the H-2 on cells such as lymphocytes does not seem to be attached to the cytoskeleton. Our own studies on lymphocytes, using the myosin affinity technique, confirm this view, most of the H-2 being apparently actin-free. Presumably, some perturbation of the H-2 itself or the plasma membrane in general must occur in the case of the P815 cell. In a companion

study, it was shown that surface components such as Ig, which are normally unattached to the actin, also form stable associations with actin filaments when they are cross-linked with antibody³⁰. A similar observation was made in another study using a different approach¹¹. Therefore, surface components such as H-2 and Ig can apparently become attached to actin when they are in a polymerised state. By analogy, it is possible that the H-2 in P815 cells is cross-linked *in situ* and becomes attached to the actin filaments associated with the plasma membrane. The focusing into the microvilli could be a simple consequence of the fact that most of the membrane-associated actin is localised in these structures. It is even possible that the two processes, attachment of H-2 to actin and the formation of microvilli, are inter-dependent, as it would serve as a device for attachment of the actin filaments to the membrane either at the initiation or the propagation stage of microvillus formation. The prevailing view is that α -actinin is involved in the attachment of the actin filaments to the membrane in the formation of microprojections. However, this has not been proved and alternative explanations cannot be dismissed. It will therefore be interesting to determine the distribution of H-2 in the microvilli.

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Cross-linked surface Ig attaches to actin

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In lymphocytes and P3 myeloma cells, cross-linking of surface Ig by the capping and patching phenomenon has been used to demonstrate the induction of a specific association between surface Ig and cellular actin.

THE redistribution of surface receptors by antibodies and lectins is thought to result from a transmembrane association

of the receptors with cytoskeletal elements in the cortex of the cell¹⁻³. Most of the evidence for this association is indirect, although there is some suggestion of a coordinated redistribution of the receptors and cytoskeletal elements⁴⁻⁶. However, it is not certain that this results from actual transmembrane associations, and alternative explanations cannot be dismissed. Evidence that there is actual binding of surface receptors to cytoskeletal elements would help to con-

solidate the transmembrane control hypothesis considerably. We describe here evidence for attachment of cross-linked surface immunoglobulin (Ig) to cellular actin.

Separation of actin

The approach used in these studies was to isolate the actin of cells and to determine whether or not the surface Ig was attached to the actin in various conditions. The surface Ig was marked by reaction with ^{125}I -labelled rabbit anti-mouse immunoglobulin antibody (RaM Ig), either as the F(ab)_2 or Fab fragments. The technique used to separate the actin from other cell components is referred to as the myosin affinity technique and relies on the strong and specific binding which occurs between actin and myosin filaments. Therefore, any actin-associated proteins might also be expected to bind to myosin filaments. That binding of putative actin-associated proteins has actually occurred through actin and not directly to the myosin can be confirmed by pre-saturating the myosin with actin, which inhibits the binding of cellular actin and its associated proteins. The technique does not distinguish between proteins which are bound directly to actin and those which bind through other proteins. The specificity of the technique for actin has been demonstrated in the preceding paper⁷. Thus, actin is the only protein or glycoprotein in a synthetic mixture to attach to myosin filaments. When NP40 lysates of lymphocytes are used, actin also binds specifically. Examination of the glycoproteins of lymphocytes shows that they have no intrinsic affinity for the myosin filaments (Fig. 1). Therefore, non-specific binding is not likely to interfere with the application of the technique to lymphocytes.

Fig. 1 Application of the myosin affinity technique to a lysate of lymphocytes. 1×10^7 lymphocytes from the lymph nodes of a BALB/c mouse were suspended in 100 μl of Tris/NP40 buffer with 1 mM phenylmethylsulphonyl fluoride (PMSF) and the nuclei removed by centrifugation. The lysate was treated with myosin filaments as described previously and analysed by sodium dodecyl sulphate (SDS) gel electrophoresis⁸. 1 And 3, lymphocyte lysate; 2 and 4, myosin filaments treated with lymphocyte lysate. 1 And 2 were stained with Coomassie blue for protein; 3 and 4 were stained for glycoproteins with ^{125}I -concanavalin A (ref. 9). The arrow shows the position of actin.

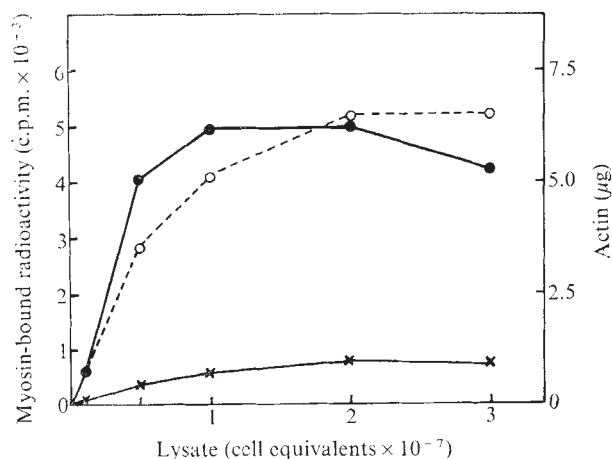
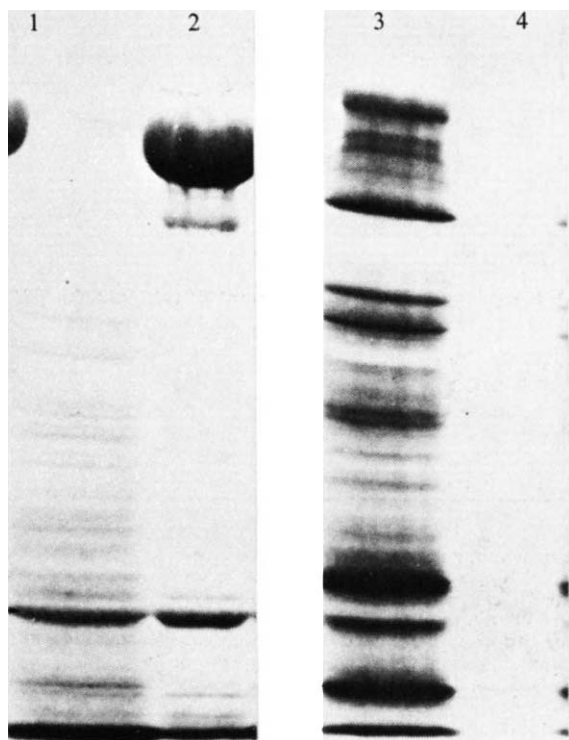


Fig. 2 Application of the myosin affinity technique to lymphocytes in capping conditions. Mouse lymphocytes were labelled with ^{125}I -RaM Ig F(ab)_2 or ^{125}I -RaM Ig Fab as described in Table 1. FITC-RaM Ig was added to the F(ab)_2 -labelled cells to monitor capping. Both sets of cells were subjected to the conditions which lead to capping described in Table 1. After this treatment the cells were lysed with 1% NP40 (see Fig. 1) and increasing amounts of the lysates mixed with myosin filaments as described in Fig. 1. The radioactivity in the washed myosin filaments was measured and the pellets subjected to SDS gel electrophoresis. Densitometry was carried out on the stained gels to estimate the amount of actin in the myosin pellets. The results obtained with both F(ab)_2 - and Fab-labelled cells were identical in this respect. ●, Actin in myosin filaments; ○, RaM Ig F(ab)_2 -treated cells; ×, RaM Ig Fab-treated cells.

Binding of surface Ig to actin

The intrinsic affinity of surface Ig for the myosin filaments was tested by labelling lymphocytes of P3 myeloma cells with Fab fragments of RaM Ig. The binding was specific, as it could be inhibited by pre-absorbing the Fab with purified mouse immunoglobulin. Nonidet P40 lysates of the labelled cells were prepared and mixed with myosin filaments. The results show that a small proportion of the radioactivity does bind to the myosin and some of this can be eliminated by using actomyosin (Table 1). In the case of the P3 cells the amount of binding in non-cross-linking conditions is very low. However, application of capping conditions, that is, divalent ^{125}I -RaM Ig F(ab)_2 as labelling reagent for surface Ig and fluorescein isothiocyanate (FITC)-RaM Ig as fluorescent reagent, had a marked effect on the binding of Ig to myosin filaments. Up to half the radioactivity in the lysates was bound to the myosin filaments and most of this was eliminated by presaturation with actin, implying that surface Ig had become attached to the actin. This was supported by studies which showed that the binding to myosin of surface Ig from capped cells followed very closely the binding profile for actin (Fig. 2). This seems to be specific for surface Ig, as other glycoproteins do not bind to myosin filaments when cells are treated with RaM Ig.

Unlike the lymphocytes, P3 cells do not undergo the capping step when treated similarly, and surface Ig redistribution is confined to patching in cross-linking conditions. However, this does not preclude binding to actin following treatment with divalent RaM Ig (Table 1), suggesting that patching is the crucial step in the attachment. This was confirmed by using lymphocytes which were prevented from capping but not patching by low temperature or by addition of sodium azide (Table 1). Both treatments effectively eliminated capping. However, attachment to actin was not prevented. This suggested that the crucial step in the attachment was the cross-linking of the surface Ig. Support for this was obtained by an experiment in which lymphocytes were labelled with ^{125}I -RaM Ig F(ab)_2 and then treated with increasing amounts of FITC-RaM Ig (Fig. 3). The

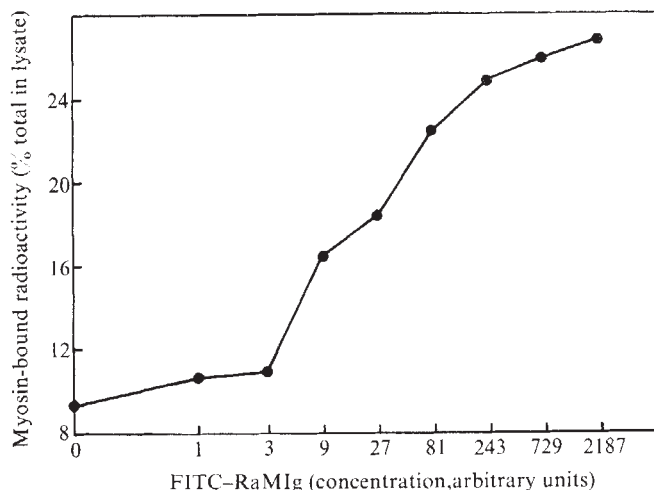


Fig. 3 Effect of cross-linking on the attachment of surface Ig to actin. P3 myeloma cells were labelled with a small amount of ^{125}I -RaM Ig F(ab) $_2$ followed by increasing amounts of FITC-RaM Ig, and incubated at 20 °C for 30 min. The patched cells were lysed with Tris/NP40/PMSF buffer and subjected to the myosin affinity technique as described in Fig. 1. The radioactivity in the myosin filaments after washing was measured to estimate the binding of the surface Ig to actin in each sample.

results show that treatment with ^{125}I -RaM Ig (ab) $_2$ causes the attachment of some radioactivity to the myosin filaments. However, as the concentration of liganded surface Ig is increased by adding cold FITC-RaM Ig, there is a further increase in the amount of radioactivity attached to the myosin filaments. Thus, increasing the degree of liganding, and presumably, therefore, of cross-linking, leads to attachment of surface Ig to the myosin filaments, suggesting that cross-linking was the crucial step in the process which leads to the attachment of surface Ig to actin. It should be noted that the possibility of the cross-linked surface Ig becoming attached to the actin after cell lysis has been examined by carrying out the lysis and myosin extraction

over a 20-fold dilution of cells. Even over such a wide range, the proportion of surface Ig which binds to the myosin filaments remains constant (data not shown), making it unlikely that the association occurs after the cells have been lysed, for in such a case the association would be expected to be dependent on the concentrations of the surface Ig and the actin. Thus, it seems that the attachment of the cross-linked surface Ig to the actin filaments takes place in the intact cell.

Interestingly the cross-linking also leads to an increase in the amount of surface Ig which associates with the nuclear pellet after detergent lysis (Table 1). However, the nuclear pellet does contain a lot of actin and this could be responsible for the phenomenon. Support for this was obtained by examining patched P3 cells which had been lysed with NP40 during examination under the microscope. The cytoplasm and membranes are dissolved away by this treatment, leaving exposed nuclei. However, the patches themselves often remain associated with a fibrillar network which envelopes the nucleus. When FITC-Fab is used the fluorescence washes away instantly. The likely explanation is that the cytoskeleton does not wash away in some cells and the attached patches remain *in situ* around the nuclei and therefore sediment with the latter.

Possible role of cross-linking in attachment

Two important questions are: is the surface Ig attached directly to the actin, and why does cross-linking lead to attachment? An answer to the former will require detailed structural analyses of the capped or patched complexes. The myosin affinity technique should prove useful in isolating this material for analysis. We would like to speculate that the cross-linking leads to attachment because of its effect on a pre-existing equilibrium direct or otherwise, between surface components such as Ig, and microfilaments. When the surface components are in a monovalent state the affinity will be low. However, an increase in the valency of one component in the equilibrium, that is, the surface component, through cross-linking, would shift the equilibrium

Table 1 Application of the myosin affinity technique to surface Ig

Cell type	Pretreatment	Incubation conditions (°C, min)	% Bound c.p.m. in lysate	% Bound c.p.m. in nuclear pellet	% Lysate c.p.m. in myosin	% Lysate c.p.m. in actomyosin
BALB/c lymphocytes	^{125}I -RaMIg Fab, 0 °C, 30 min	1.20, 30	81	19	10	5
		2.20, 30	82	18	10	4
		10 mM azide				
	^{125}I -RaMIg F(ab) $_2$ FITC-RaMIg 0 °C, 30 min	3.0, 30	82	18	10	5
		1.20, 30	32	68	42	4
		2.20, 30	39	61	50	3
P3 myeloma cells	^{125}I -RaMIg Fab, 0 °C, 30 min	10 mM azide				
		3.0, 30	46	54	38	3
		20, 30	92	8	2	2
	^{125}I -RaMIg FITC-RaMIg 0 °C, 30 min	20, 30	60	40	54	0.7

Lymphocytes were obtained from peripheral lymph nodes of BALB/c mice and washed 3 times with Hank's balanced salts solution and 1 mM HEPES buffer, pH 7.3 (BSS/HEPES), before use. P3 Myeloma cells were grown in culture and treated as above before use. Labelling of surface Ig with ^{125}I -RaM Ig Fab or ^{125}I -RaM Ig F(ab) $_2$ was carried out on ice for 1 h in BSS/HEPES. Specificity of the RaM Ig antibody and its fragments for surface Ig was checked by preabsorbing with purified IgG from P3 cells. Redistribution of surface Ig was effected by treating cells with FITC-RaM Ig on ice for 30 min and raising the temperature to 20 °C for 30 min to induce capping. In the case of lymphocytes about 30% of the cells were labelled by the FITC-RaM Ig and over 80% of these were capped. P3 Cells were completely labelled by FITC-RaM Ig but redistribution was confined to patching. For use in the myosin affinity technique labelled cells ($0.5-1 \times 10^7$) were pelleted and made up in 200 μl of BSS/HEPES with 1% Nonidet P40 + 1 mM phenylmethylsulphonyl fluoride on ice, and the nuclei removed by centrifugation at 400g for 10 min. The lysates were diluted 3 times with distilled water and treated with myosin filaments as described in Fig. 1 before gel electrophoresis to confirm the binding of actin to the myosin filaments. ^{125}I -radioactivity in the treated myosin filaments was measured to quantitate the binding of surface Ig. Presaturated actomyosin was prepared as described in Fig. 1 and used exactly as above.

towards attachment and lead to the observed effect. Recent studies on the attachment of histocompatibility antigens to cytoskeletal elements also suggest that cross-linking of the surface component is the signal for attachment to the cytoskeleton¹⁰. Thus, it is conceivable that cross-linking of surface receptors of many types could have an important role in the transmission of transmembrane signals in general.

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letters to nature

Circumstellar methane in the infrared spectrum of IRC+10°216

WE reported previously^{1,2} the detection of lines due to circumstellar CO, C₂H₂ and HCN in the infrared spectrum of the cool carbon-rich object IRC +10°216. In this letter we announce the further detection of lines due to circumstellar CH₄ in 3.3- μ m spectra of this source.

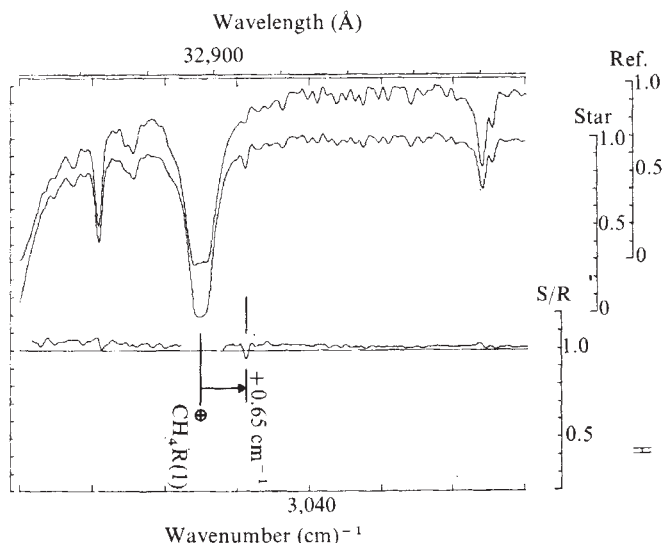
A prototype, rapid-scanning Fourier transform spectrometer^{3,4} at the coude focus of the Mayall 4-m telescope was used to obtain a high resolution (0.05 cm⁻¹) spectrum of IRC+10°216 within the 2,840–3,075 cm⁻¹ bandpass of a cooled interference filter. This spectral interval encompasses the ν_3 fundamental band of CH₄ which produces saturated absorption lines in the terrestrial atmosphere. As the temperature of the circumstellar material enshrouding IRC+10°216 is comparable with that of the Earth's atmosphere, the circumstellar lines are only detectable from the ground when they are sufficiently doppler shifted. When the source was observed on 21 November 1977 the -35 km s⁻¹ heliocentric radial velocity of the circumstellar lines was complemented by a further -30 km s⁻¹ due to the Earth's orbital motion. The resulting 0.65 cm⁻¹ blueshift was sufficient to displace many low excitation lines into regions of high atmospheric transmission (Fig. 1). The R(0), R(1), R(3) and Q(1) lines are particularly clean and give a mean heliocentric radial velocity of -34.1 ± 0.5 km s⁻¹, in excellent agreement with the -32.5 ± 0.2 km s⁻¹ determined for CO lines of similar excitation energy (in preparation). In the case of higher excitation lines, fine structure produces broad manifolds⁵ and only occasional components are distinguishable.

The line strengths measured by Toth *et al.*⁶ have been used to derive a column density $N(\text{CH}_4) = 2.5 \pm 1.0 \times 10^{17}$ cm⁻² from the measured equivalent widths of low excitation lines. The quoted error is due to poor definition of the circumstellar temperature profile and could be greatly reduced by careful modelling. Corresponding values for other molecules are $N(\text{CO}) = 1.0 \pm 0.4 \times 10^{20}$ cm⁻², $N(\text{C}_2\text{H}_2) \gtrsim 3 \times 10^{19}$ cm⁻² and $N(\text{HCN}) \gtrsim 1.5 \times 10^{18}$ cm⁻². The last two values are poorly determined because the lines in the 3.1- μ m bands are too saturated to use and laboratory determinations of the strengths of weak combination bands of C₂H₂ ($\nu_1 + \nu_5$ at 4,091 cm⁻¹) and HCN ($\nu_2 + \nu_5$ at 2,701 cm⁻¹ and $\nu_2 + \nu_3$ at 4,004 cm⁻¹) are relatively crude. It seems clear that improvements of these determinations and careful modelling

of the source would provide stringent tests of the chemical equilibrium in the circumstellar material obscuring IRC+10°216.

Most of the circumstellar absorption arises in a region characterised by $n \sim 10^8$ cm⁻³ and $T \sim 400$ K. In these conditions molecular abundances are not likely to change rapidly. Therefore, it may be appropriate to interpret the column densities with respect to conditions in the outer layers of the stellar photosphere. We have compared observed abundances to Tsuji's⁷ calculations of the relative abundances of molecules formed from H, C, N and O in the atmospheres of carbon rich supergiants with $n \sim 10^{14}$ cm⁻³, $T \sim 1,000$ K. The observed relative column densities of CO, C₂H₂, HCN and CH₄ are in striking agreement with his predictions of the relative abundances for the

Fig. 1 The spectrum of IRC+10°216 in the region of the CH₄ R(1) lines. Upper trace, a comparison solar spectrum (dominated by terrestrial absorption); middle trace, the +10°216 spectrum; lower trace, the ratio +10°216/sun. A 2 σ error bar is shown at the lower right. The resolution is 0.05 cm⁻¹ and the FWHM of the instrumental line profile is 0.06 cm⁻¹. The doppler shift between the terrestrial and circumstellar lines as predicted from measurements of low excitation circumstellar CO lines is indicated.



case where there has been no appreciable precipitation of carbon as graphite and are at marked variance with the predictions if substantial graphite precipitation is permitted. Graphite is thought to be the dominant source of circumstellar extinction in IRC+10°216 and detailed modelling⁸ requires an optical depth ~ 6 corresponding to a precipitate column with $N(C) \sim 6 \times 10^{19}$. It remains to be seen whether precipitation of this much carbon as graphite is consistent with the observed molecular column densities.

Although there is considerable circumstantial evidence for the presence of CH_4 in molecular clouds and recent theoretical work suggests that CH_4 may also occur with relatively high abundance in some interstellar environments⁹, confirmation at radio frequencies has been hampered by the lack of strong transitions there. It now seems that infrared spectroscopy at 3.3 and 7.8 μm can be used to measure CH_4 abundances in molecular clouds obscuring both young and evolved stellar objects.

All lines within our 2,840–3,075 cm^{-1} bandpass are identified as methane. The absence of any other features with equivalent widths exceeding the 3σ noise value of 0.002 cm^{-1} enables stringent upper limits to be placed on the column densities of other hydrocarbons which have strong bands in this region. These limits imply that emission by such molecules in the 10–13 μm range is at best several orders of magnitude too small to account for the observed emission feature¹⁰. This is contrary to the speculation of Tarafdar and Wickramasinghe¹¹ that C_nH_{2n} molecules might account for the 10–13- μm emission peak.

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Origin of the grooves on Phobos

THE characteristic surface features of the inner martian satellite Phobos are impact craters and long linear depressions, here called grooves. The grooves have been explained as surface manifestations of deep-seated fractures, but there has been a divergence of opinion as to the cause of the fracturing. Soter and Harris¹ have attributed the fracturing to tidal stresses induced by Mars; Pollack and Burns² have suggested fracturing by drag forces during the hypothetical capture of the satellite by Mars; Veverka *et al.*³ have attributed it to a large, nearly catastrophic cratering

event. We present here new data on the grooves which tend to support the cratering hypothesis and seem to rule out the tidal mechanism.

Although it was known⁴ from the Mariner 9 images obtained in 1971–72 that the surface of Phobos is heavily cratered, the discovery of the grooves by the Viking Orbiters came as a surprise. An effective resolution of < 70 m is needed to see grooves well, whereas the best resolution achieved by Mariner 9 was > 200 m. Sufficient Viking Orbiter imagery has now been obtained to map the surface distribution of grooves and study their morphology, and to date them by means of the density of superimposed impact craters.

Figure 1 is a map of the distribution of grooves on Phobos. For reference we have sketched in the outline of the largest topographical feature on Phobos—the 10-km crater Stickney. The region west of Stickney, between longitudes 70° and 160°W, has been imaged at resolutions of only > 150 m, whereas imagery with a resolution of ≤ 50 m exists for the remainder of the satellite. Thus, no significance should be attached to the apparent low density of grooves west of Stickney evident in Fig. 1.

The striking pattern in Fig. 1 is that the grooves emanate from all sides of Stickney and run continuously towards a region near 270°W where they die out. This area, although not exactly 180° away from the centre of Stickney, is on line with a surface normal from its centre when one considers the actual three-dimensional shape of Phobos. (Phobos⁵ is approximately a triaxial ellipsoid with principal axes $27 \times 21 \times 19$ km across; the longest axis coincides with the 0°–180° longitude direction.)

The remarkable pattern of grooves seen in Fig. 1 strongly suggests that the formation of Stickney and of the grooves were intimately related events. This idea is supported by the typical morphology of the grooves. Individual grooves are best developed in the neighbourhood of Stickney and taper out near longitude 270°. Most grooves are 100–200 m wide and 10–20 m deep, with concave-up cross-profiles. The largest grooves, 700 m wide and 90 m deep, are near Stickney. Only near the antipodal point near longitude 270° are the grooves consistently less than 100 m in width.

The grooves have a more complex morphology than that expected of simple fractures. They commonly have a beaded or pitted appearance due to subtle depressions along their lengths. Some grooves, especially between longitudes 170° and 220°W have wide, irregularly bounded segments of hummocky topography, some of which seem to rise above the surrounding surface. Some straight-walled groove segments seem to have subtle, slightly raised rims. Thus, the morphology of the grooves is not that of simple fractures or of fault blocks. The grooves almost certainly represent some response of the Phobos regolith to deeper fractures, and it is probable that the formation of the grooves involved a variety of processes of which deep-seated fracturing was dominant. Attempts to elucidate some of these processes are underway⁶. Whatever the modifying processes, the grooves seem to be closely related to the formation of Stickney.

The orientations of most grooves are consistent with the concept of planar fractures cutting through much of Phobos. One prominent set of groove planes is nearly parallel to the equatorial plane of Phobos, a second is roughly perpendicular to the sub-Mars direction, and a third is intermediate between the first two, with southern hemisphere normals on the Mars side of Phobos. Although the grooves are not radial to the rim of Stickney, the major planes defined by the grooves are roughly perpendicular to the plane of the crater's rim.

The age of the grooves can be estimated from the density of superimposed impact craters (Fig. 2). In Fig. 3 we compare the density of impact craters within the grooves with the average value for all of Phobos. Also shown for com-

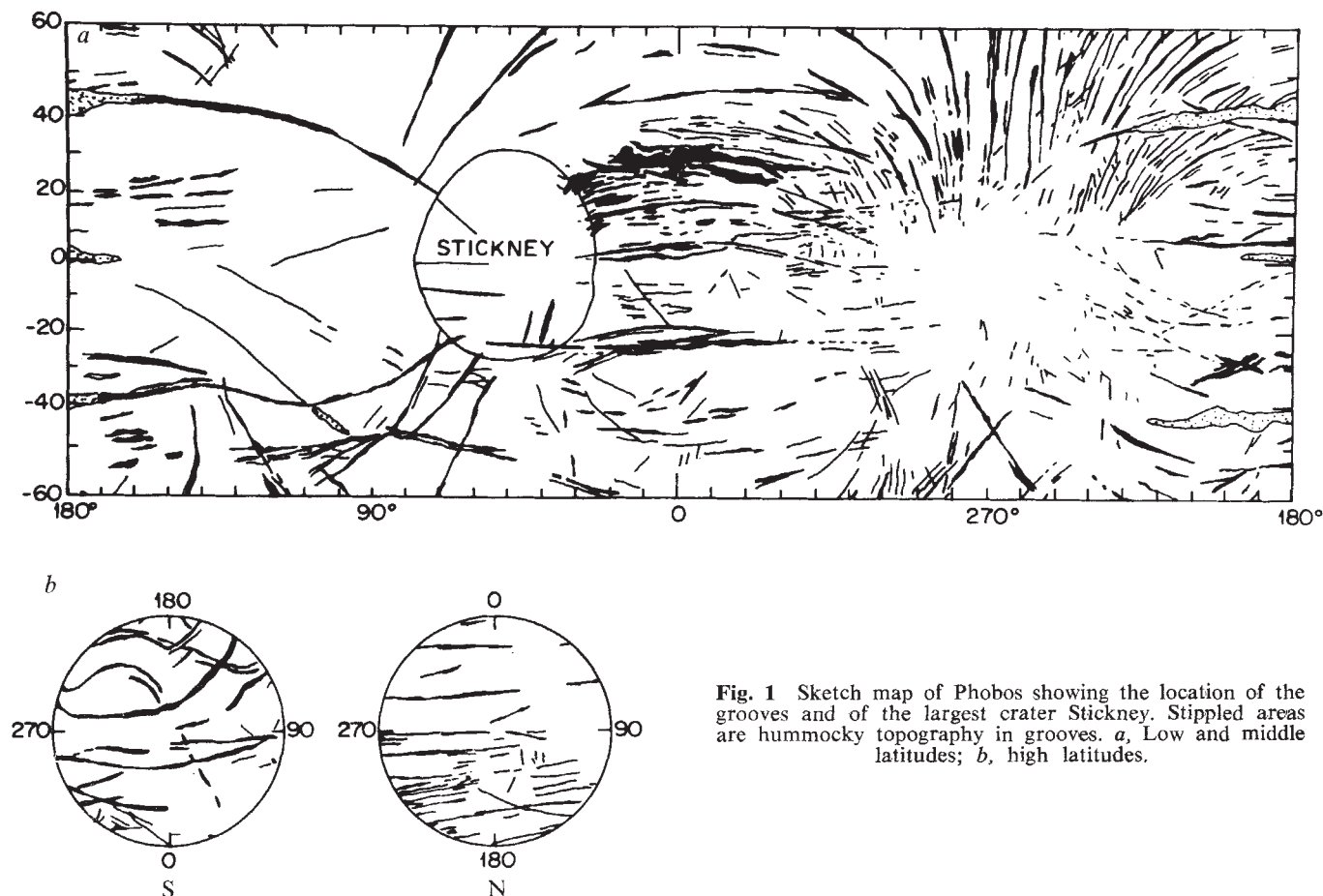


Fig. 1 Sketch map of Phobos showing the location of the grooves and of the largest crater Stickney. Stippled areas are hummocky topography in grooves. *a*, Low and middle latitudes; *b*, high latitudes.

parison is an extrapolation of the crater density curve for the lunar uplands⁷. Within the error bars, we consider the density of impact craters averaged over Phobos to be equal to the extrapolated value for the lunar uplands, and the density within the grooves to be at least half this value. On this basis, the absolute age of the grooves can be estimated using the techniques of Hartmann⁸. A crater production rate for Mars twice that of the Moon, scaled to the g on Phobos ($g \approx 0.5 \text{ cm s}^{-2}$; crater diameters scaled as $g^{-0.12}$, ref. 9) gives an age in excess of $3.4 \times 10^9 \text{ yr}$ for the grooves. Extreme values of fluxes and of gravity scaling exponents must be used to yield ages less than 10^9 yr .

The old age of the grooves, their pattern on the surfaces, as well as their evident association with the crater Stickney, are not consistent with the tidal hypothesis of Soter and Harris¹. If martian tides were the main cause of grooves, the grooves should be younger than 10^9 yr as Phobos is presently spiralling into Mars on a timescale of 10^8 yr (ref. 10).

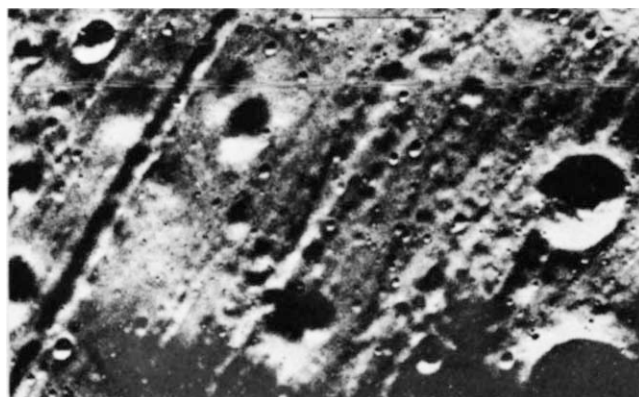
The fracturing of Phobos by drag forces during its hypothetical capture by Mars does not explain the evident association of grooves with the crater Stickney.

The old age of the grooves and their pattern on the surface of the satellite are best explained by the third hypothesis, according to which the grooves are modified surface expressions of deep-seated fractures produced during the formation of Stickney. Available evidence suggests that Stickney is of comparable age to the grooves, and Pollack *et al.*⁴ have calculated that the energy of impact that formed Stickney may have been within an order of magnitude of the binding energy of Phobos. It has recently been suggested¹¹⁻¹³ that Phobos is made of a mechanically weak material similar to that found in primitive carbonaceous chondrite meteorites. Thus, it seems plausible that a severe

impact such as that responsible for the formation of Stickney could fracture much of the satellite.

According to our argument, other small bodies in the Solar System made of similar mechanically weak material (some C asteroids, for example¹⁴) may be expected to have similar patterns of grooves associated with the largest impact craters. Images of slightly more than half of Deimos obtained during the October flyby do not show any grooves. The absence of grooves on Deimos might be explained by the lack of a crater large enough to cause global fracturing. The largest crater seen so far is only about 3 km across.

Fig. 2 Part of Viking Orbiter picture 246A06 showing impact craters within the grooves. The scale bar represents 1 km.



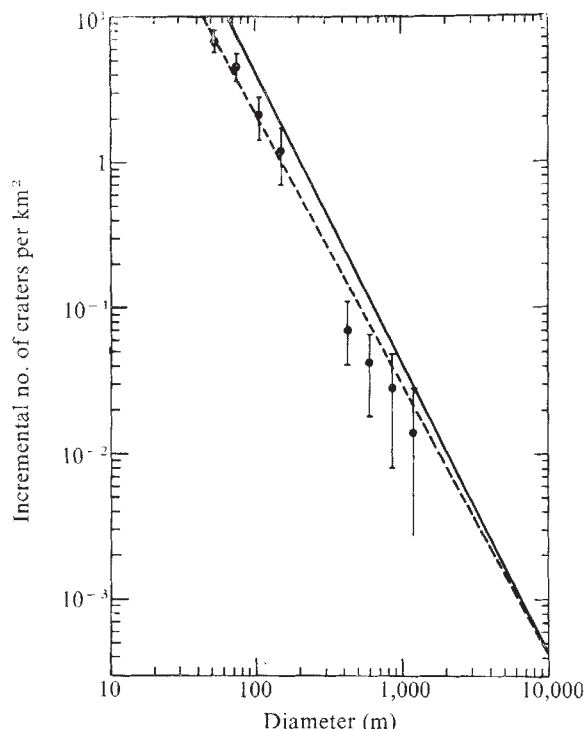


Fig. 3 Surface density of impact craters within the grooves (●) compared with that on the rest of Phobos (—) and on the lunar uplands (—). The lunar curve is extrapolated from Hartmann⁷. Within the accuracy of the data, we consider the dashed and solid curves to be identical. The data points for the grooves fall into two groups, as two sets of images of differing resolution were used.

It is also possible that Deimos does not have the same composition as Phobos and might therefore have different mechanical properties.

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Major satellites cause wavy deformation of Saturn's rings

THE rings of Saturn consist of many solid particles, each revolving around Saturn as an independent satellite. Typical particle size or, more exactly, mean particle radius, ρ , has been estimated from radio interferometry and radar observations to be 2–15 cm (refs 1, 2)—consistent with a ring thickness of just over 10 m. However, the ring thickness z_0 , as determined photometrically is at least 4 orders of magnitude greater, at 1–3 km (refs 3, 4). The generally-accepted explanation for this difference is that the particle orbits are not fully coplanar, but inclined to the mean ring plane at appreciable angles i_p . However, such a model would be unstable. Indeed, particle collisions must be frequent as each particle crosses the mean plane twice for one revolution and the probability of meeting another particle is high. As a result of collisions, the energy of particle movement normal to the mean plane is dissipated, and so very soon the particle orbits must become coplanar, that is $z_0 \rightarrow 2\rho_{\max}$ where $\rho_{\max}$ is very likely much less than 1 km. I show here that allowing for gravitational perturbations of particle orbits by satellites and the oblateness of Saturn one may obtain a model satisfying the observational data. The main features of the model are: (1) specific wavy deformation of the rings which thus appear twisted—this is the consequence of differential precession of particle orbits with $i_p \neq 0$; (2) particle collisions are rare (and there is therefore only slow energy dissipation) because the neighbouring particles move in nearly the same phase.

Consider particle perturbations caused by those of Saturn's satellites with orbits outside the mean ring plane. Their perturbing force will cause the particle orbits to go out of this plane. The movement of a particle along such an orbit has a component normal to the mean plane, with an amplitude A_z . The most important perturbing body is the largest of Saturn's satellites, Titan, in spite of its small inclination $i = 0.3^\circ$. According to calculations carried out by Franklin⁵, Titan, after 40 revolutions (≈ 640 d), would give rise to a value for A_z of ~ 300 m.

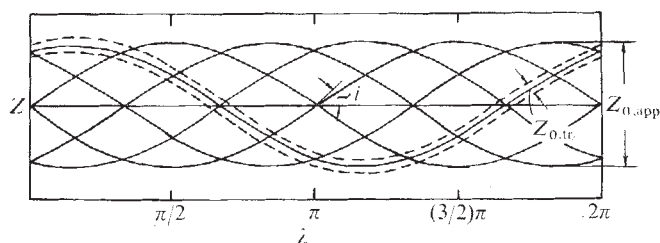
These perturbations are of long periodicity. It follows from the above considerations, that full development of A_z requires either the absence of, or weakness of particle collisions. This condition is met by the perturbation of adjacent particles in nearly the same phase. An estimate of the time interval between the successive collisions of a given particle is presented below.

Another effect to be considered is the perturbation by Saturn's oblateness. It results in precession of particle orbits (nodes and apsides) with the rate⁶:

$$|\dot{\omega}| = J(\gamma M)^{1/2} r_0^2/a^{7/2} \quad (1)$$

J being the parameter expressing Saturn's oblateness, γ the gravitational constant, M and r_0 the mass and equatorial radius of Saturn, and a the semimajor axis of the particle orbit. I

Fig. 1 'Particle chains' of Saturn's rings displayed in coordinates λ (saturnicentric longitude) and z (distance from Saturn's equatorial plane); i = inclination of chains due to perturbations by the satellites; $z_{0,app}$ and $z_{0,tr}$ denote the apparent and true thickness of the rings respectively.



neglect here the eccentricity and inclination of the orbit. Note that $|\omega|$ depends on a ; that is, the precession is differential.

To visualise the disturbed figure of the rings, it is convenient to adopt the assumption that all the particles have the same radius ρ and are grouped in concentric, nearly circular chains, l being the distance between the centres of neighbouring particles and i_p the inclination of the chain. Because of the differential precession, the longitude λ of the chain node depends on the value of its semimajor axis a . Therefore at any given moment the nodes of various chains will have all possible values of λ (Fig. 1).

Let us assume that Saturn's rings are edge-on to the Earth (the position in which an estimate of thickness is possible). An observer of the rings will estimate their apparent thickness $z_{o,app}$ as $2A_z$. (I omit here for simplicity the dependence of A_z on a and λ). At the same time, the true ring thickness, $z_{o,ir}$, measured in a region small in comparison with the rings' radial width, and determined by the values of ρ_{max} and residual oscillations of particles about their mean positions, is much lower than $z_{o,app}$. The rings are thus not plane-parallel, but twisted.

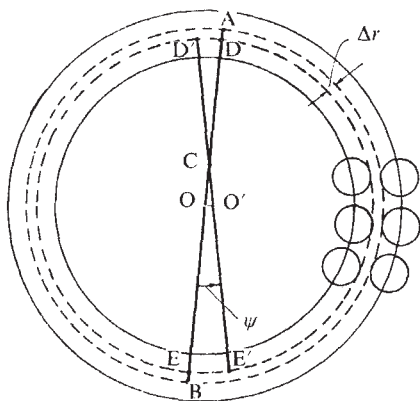


Fig. 2 Schematic view of the two adjacent particle chains. The centre of Saturn's mass is in C. The lines of apsides AB and DE, initially coinciding with each other, after some time will form an angle ψ . When $00' = \Delta r$, the collision of particles is possible.

As mentioned above, particles close to each other should be disturbed in nearly the same phase. Therefore they cannot collide until they accumulate sufficient phase difference at the expense of differential precession. As a result, the energy gained by particles from perturbations dissipates very slowly. The concept of particle chains leads to a method of estimating the minimum time interval, Δt between two successive collisions of a pair of particles belonging to adjacent chains (Fig. 2). Assuming a particle radius $\rho = 15$ cm, $l = 2.5\rho$ (that is, optical thickness of the rings $\tau_o = 0.7$) and the eccentricity of chains $e = 10^{-3}$ we find from equation (1) that for the outer edge of the ring system (where A_z is maximum), $\Delta t = 1,200$ d—much longer than the time needed for full development of A_z from Titan. In addition, simple calculations show that the relative velocity of colliding particles normal to the ring plane is only 10^{-6} cm s $^{-1}$. The energy dissipation through such rare and weak collisions cannot prevent the full development of 'twisting' perturbations in spite of their long periodicity.

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Simultaneous observations of equatorial ionospheric scintillation on four frequencies

RADIO waves emanating from stars or satellites fluctuate in intensity when received on the ground. This phenomenon, radiosintillation, arises from irregularities in the ionosphere through which the waves must pass. These scintillations have been studied in an attempt to understand the properties of the irregularities themselves but with the advent of satellite communications the knowledge of the ionospheric scintillations has become essential for the proper planning of the systems. One of the important unresolved problems is the variation of scintillation with frequency. This note describes our recordings of the scintillations at equatorial latitudes occurring simultaneously on 40, 140, 360 and 860 MHz.

Observations of radio stars over the frequency range 26–208 MHz have shown that the mean scintillation amplitude varies as the square of the wavelength up to the point where the fluctuations on the lower frequency saturated¹. Briggs and Parkin² showed that the scintillation depth (S) can be expressed in the form $S \propto \lambda(\sec \theta)^{1/2} / [1 + (\pi^2 r^4) / (4\lambda^2 z^2)]^{1/2}$ where z is the distance of the irregularity, r the irregularity auto-correlation distance in the medium, θ the zenith angle at the ionospheric intersection point and λ the wavelength. For small irregularities, a long wavelength or large slant distance ($r^2 \ll \lambda z$) the scintillation index (S) would be proportional to wavelength (λ). For large irregularities, however, a small wavelength or small slant distance ($r^2 \gg \lambda z$), $S \propto \lambda^2$. Aarons *et al.*³ described the frequency variation of the scintillation by $S = kf^{-n}$, where k is a constant and n the 'spectral index' of the scintillations (we shall refer to it here as the exponent to avoid confusion with the scintillation index). For the frequency range 113–400 MHz the exponent was found to be $n = 2$ for weak scattering. At the lower frequencies (30–113 MHz) the n was generally lower between 0 and 1. Simultaneous observations on 137 and 412 MHz by Whitney *et al.*⁴ estimated the value of n to be close to 1.5.

The launching of the ATS 6 geostationary satellite with radio beacons on 40, 140 and 360 MHz was an excellent opportunity for studying the frequency dependence of the ionospheric scintillations. In the second phase of its operation ATS 6 was shifted to 35°E long which enabled Indian scientists to study the scintillations within the strong scintillation zone near the magnetic equator. Apart from these beacons, there was a television downlink on 860 MHz for the Satellite Instructional Television Experiment (SITE). However, because of restrictions in the power available aboard the satellite, the beacons on 40, 140 and 360 MHz were in general switched off during the periods of 860 MHz transmissions.

Krishna Murthy *et al.*⁵ have described the preliminary results of scintillation of ATS 6 signals at Trivandrum (dip 0.6°S). They suggested that the S_4 index of scintillation can be divided into two groups depending upon whether this index at 360 MHz is greater or less than ~ 0.5 . Two values of the exponent, $n = 1.2$ for frequencies ≥ 140 MHz and 0.46 for the lower frequencies, were derived. However, their data were not obtained on all frequencies simultaneously and it is therefore possible that the exponents determined may have been affected by the temporal variation of the scintillation depth.

The ATS 6 signals on all frequencies were recorded at Ootacamund (dip 4°N) and some features of scintillations on 40, 140 and 360 MHz have been described elsewhere⁶. The scintillations were most predominant between 2000 LT and 2300 LT

and were attributed to the spread F condition. It was shown that the exponent, n , for a particular set of frequencies was not constant but decreased with increasing magnitude of scintillations. For weak scintillations the exponent was found to be close to 1.0. At Ootacamund, the amplitude of the carrier wave of the TV transmission on 860 MHz was also recorded. For 2 weeks around the eclipse day, 18 April 1976, the radio beacons on 40, 140 and 360 MHz were kept on simultaneously with the TV transmission on 860 MHz and on two of the evenings the scintillations were observed over the entire frequency range. Figure 1 shows records of ATS 6 signals on different frequencies received at Ootacamund on 19 and 20 April 1976. On 20 April relatively weak scintillations were observed on 860 MHz and the scintillations of other signals are seen to increase with decreasing frequency. On 19 April, strong scintillations were noticed on 860 MHz, so that the picture on the TV screen was

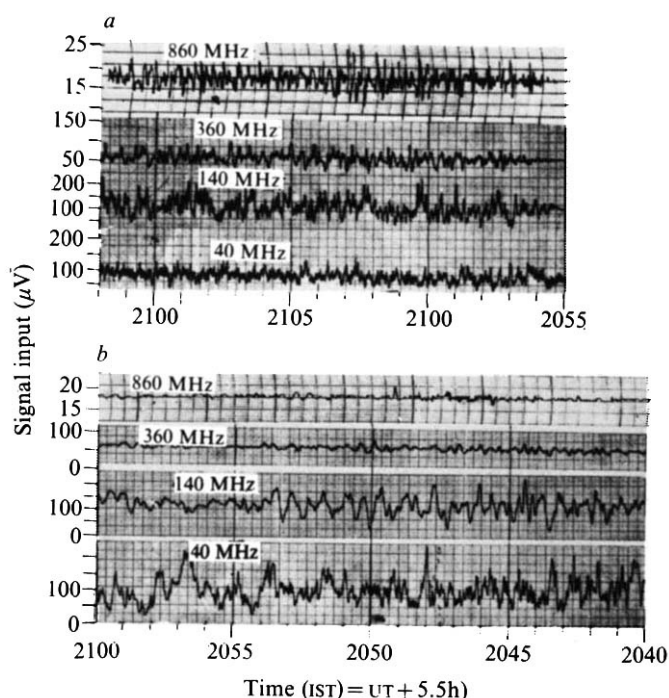


Fig. 1 The amplitude fading records of radio beacons on 40, 140, 360 and 860 MHz from ATS 6 (34°E) satellite received at Ootacamund on a, 19 April and b, between 2055 and 2110 IST; 20 April 1976, between 2040 and 2100 IST.

very unstable. The scintillations seem to increase on 360 and 140 MHz but at the lowest frequency (40 MHz) the scintillation decreases. These records were scaled at an interval of 15s and the average scintillation index S_4 , defined from $(S_4)^2 = (R^2 - \langle R \rangle^2) / \langle R^2 \rangle$ with R the instantaneous amplitude, computed for the entire period. Figure 2 shows these data plotted against frequency on a double logarithm scale. If S_4 is proportional to f^{-n} then the points for different frequencies will lie on a straight line of slope $-n$.

For the weak scintillations on 20 April, with an S_4 at 860 MHz of 0.05, it can be seen that the points for the three upper frequencies lie approximately on a straight line of gradient of magnitude 1.2. For the 40/140 MHz frequency pair the exponent is 0.40. However, if the exponent, n , was to remain constant at 1.2, even with the S_4 at 860 MHz as low as 0.05, the S_4 would exceed 1.0 at 40 MHz which is not practicable. Thus, the exponent, n , decreases beyond a threshold of scintillation amplitude when scintillations should be considered as strong.

For the strong scintillations on 19 April, with an S_4 at 860 MHz of 0.24, n for 860/360 MHz is 0.67 and for 360/140 MHz

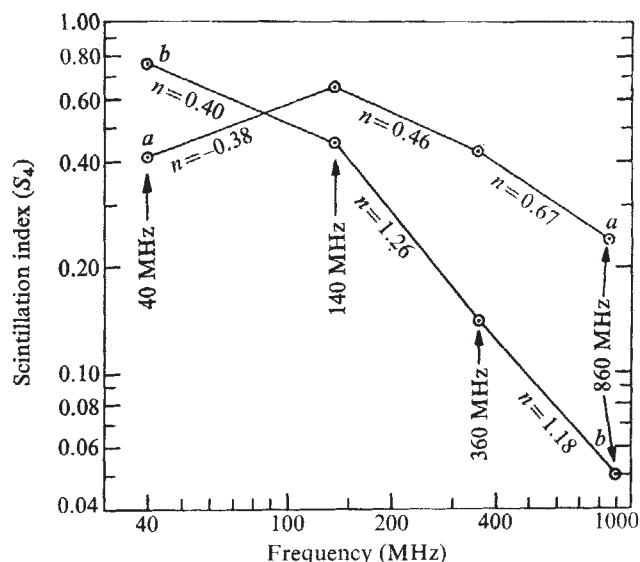


Fig. 2 The frequency variation of the index S_4 of ionospheric scintillations observed simultaneously on 40, 140, 360 and 860 MHz signals from ATS 6 satellite received at Ootacamund. a, 19 April; b, 20 April 1976.

0.46. The average exponent over these three frequencies is 0.55. It is interesting that S_4 at 40 MHz was 0.4 which is less than the corresponding S_4 at 140 MHz giving a negative value of the index n ($= -0.38$). Thus, in the case of strong scintillations n not only decreases towards lower frequencies but can also become negative (see Table 1).

Rufenach⁷ has calculated the spectral exponent for power law irregularities and has found $n \sim 1.4$ to 1.6 for small scintillation levels whereas according to Briggs and Parkin⁸ for gaussian law irregularities n should be equal to 2.0. Yeh *et al.*⁸ have considered (theoretically) the effect of multiple scattering on the ionospheric scintillations and have shown that the value

Table 1 Effect of frequency on the scintillation exponent

Frequency (MHz)	Exponent (n)	Conditions	Refs
113-400	2	Weak scattering	3
30-113	0.1		4
137-412	1.5		
140	1.2	Weak scintillation	5
140	0.46		6
40-360	1		
140-860	1.2	Weak scintillation on 360 MHz	
40-140	0.4		
140-860	0.55	Strong scintillation on 360 MHz	}
40-140	-0.38		

of $n = 1.5$ at higher frequencies and $n = 1$ for lower frequencies.

The present results although only on a limited sample indicate that within the equatorial F region, irregularities obey the power law distribution, with the spectral exponent of scintillation close to 1.3 for higher frequencies and for weak scintillation levels. However, n decreases with increasing magnitude of the scintillation and may become negative in the case of strong scintillations at the lower frequencies.

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Three-dimensional storm motion detection by conventional weather radar

KNOWLEDGE of the kinematic structure of storms is important for understanding the internal physical processes. Radar has long provided information on the three-dimensional structure of storms from measurements of the radar reflectivity factor alone. Early users of radar gave total storm movement only, whereas later radar data were used to reveal internal motions based on information related to cloud physics such as the three-dimensional morphology of the storm volume. Such approaches have continued by using the increasingly finer scale details provided by more modern radar systems. Both Barge and Bergwall² and Browning and Foote³ have used fine scale reflectivity structure to determine airflow in hailstorms. Doppler radar added a new dimension to our capabilities through its ability to measure directly the radial component of motion of an ensemble of hydrometeor particles. Two⁴ or three⁵ Doppler radars collecting data in conjunction, the equation of mass continuity, and an empirical radar reflectivity-terminal velocity relationship have enabled the estimation of the full three-dimensional airflow fields in parts of storms. Because

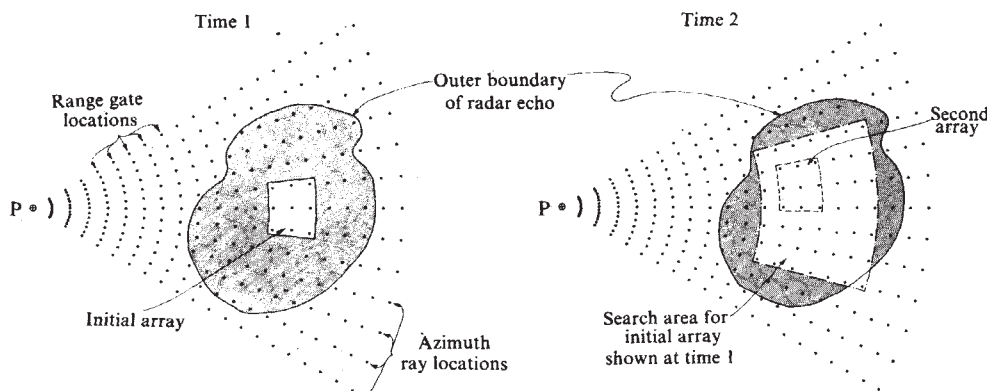
of the inherent advantage of Doppler radar in motion detection, little effort has been directed toward developing objective schemes of determining internal storm motions with conventional meteorological radars. Pattern recognition schemes using correlation coefficient techniques⁶, Fourier analysis⁷, and gaussian curve fitting⁸ have been used with radar and satellite data, but primarily for detecting overall storm motions, echo merging and echo splitting. Here we describe an objective use of radar reflectivity factor data from a single conventional weather radar to give information related to the three-dimensional motions within a storm.

The basic technique uses the correlation coefficient approach for pattern recognition. An initial array of radar reflectivities is correlated with later arrays. By locating the second array with the best correlation, a motion vector for the initial array is determined. This objective tracking of radar echo with correlations (TREC) is done by computer.

Consider, for example, the storm depicted in Fig. 1. The storm is scanned in azimuth by a radar located at P with measurements of storm intensity made at range rates along each given radial (azimuth ray). Consider an array of radar reflectivity factors n range gates long and n azimuth rays across at time t_1 , lying on a conical surface. Some time later (t_2) we can compare our initial array of reflectivities with a second array located on the same surface, and calculate a correlation coefficient. We then shift this second array and repeat the calculation. This procedure continues until all possible second arrays have been searched over a specified area, the size of which is based on the known storm velocity, rawinsonde wind data or some other criterion. An arrow is then drawn from the centre of the initial array to the centre of the best correlated second array, provided the radar reflectivity factor at each centre location exceeds a pre-selected reflectivity. In our case we require a reflectivity of $10(zdB_z \equiv 10 \log z \text{ (mm}^6 \text{ m}^{-3}\text{)})$. Motion vectors at other locations are determined by systematically examining initial arrays throughout the storm and repeating this entire correlation procedure for each.

Figure 2 shows the results of this procedure applied to a hailstorm observed near Grover, Colorado, on 22 June 1976 by the S-band, narrow-beam radar of the National Hail Research Experiment. Data were collected starting at 1625 MDT for the initial arrays and 1627 MDT for the second arrays, both at an elevation angle of 3.3° . Because of known storm motions on this day (average cell speed of 14 m s^{-1}), the search area was restricted to ± 5 gates and ± 5 rays from the initial position. Five rays may be too few near the radar and too many far from the radar, so in the future a specified size (in kilometres) of the search area is planned. Initial arrays were 15 gates long by 15 rays across and are spaced 5 gates and 5 rays apart. Thus, adjacent points in Fig. 2 have a 2/3 overlap with immediate neighbours, 1/3

Fig. 1 Representation of a storm scanned by a radar located at P, where a measurement of radar reflectivity factor is made at each range gate/azimuth ray location. The initial n by n array at time 1 (where $n=3$ here) is compared with all n by n second arrays in the search area at time 2, where one of these second arrays is depicted.



overlap with their second nearest neighbours, and are completely independent of their third nearest neighbours. Figure 3 shows the flow field 2 min later. While there are some differences, the general features are quite consistent from one time to the next. Similar continuity is evident when looking at the patterns from one tilt angle to the next.

Several points can be made about the results in Figs 2 and 3. First, the procedure detects tangential motions as easily as radial. This should be true in the vertical as well as the horizontal. Second, the total area covered is fairly large, approximately five times that of the triple-Doppler system collecting data on the same storm at the same time. Third, the procedure can sometimes give vectors which are inconsistent. An example of this is the pair of crossing arrows in Fig. 2, 50 km east and 28 km north of the radar. Erroneous vectors might be eliminated by using the rate of change of available reflectivity information, by requiring that the correlation coefficients exceed some value, or by some other optimisation procedure which can be applied to TREC.

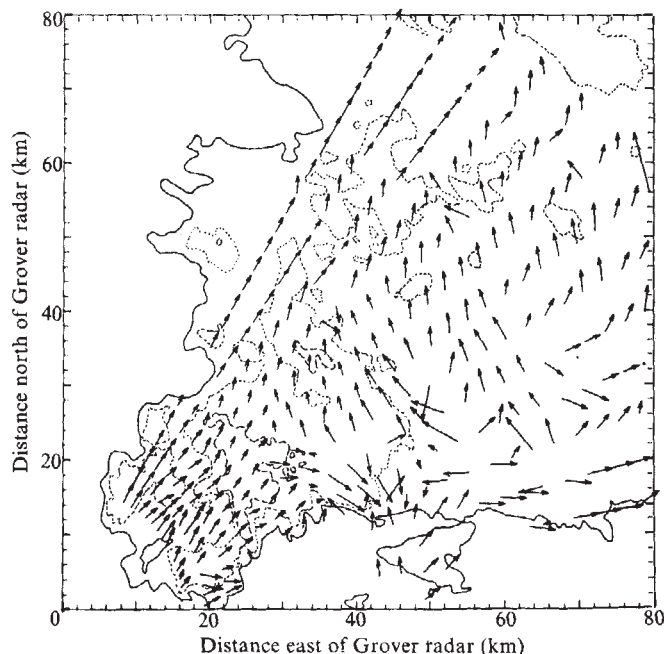


Fig. 2 Reflectivity motion field for data collected on 22 June 1976 at 1625 MDT and 1627 MDT at an antenna elevation angle 3.3° above the horizon. Dots indicate that the initial and final arrays were best correlated at the same locations. Superimposed are the 15, 35 and 55 dB_z radar reflectivity contours for the 1625 MDT data.

Tracking reflectivity patterns may not give the same information as the direct measurement of airflow. A triple-Doppler radar system does yield airflow and can be compared with the results of TREC. Such a system, operated jointly by NCAR and the Wave Propagation Laboratory of the National Oceanic and Atmospheric Administration, was collecting data on the same storm system. None of the three Doppler radars used was located at the Grover radar site. Also, the output of the triple-Doppler processing is on horizontal (or vertical) planes while TREC processing is on constant tilt conical surfaces. As the elevation steps used by the Grover radar were quite large on this day, conical surfaces were reconstructed from the Doppler data, rather than by attempting to construct constant altitude surfaces from the Grover radar data.

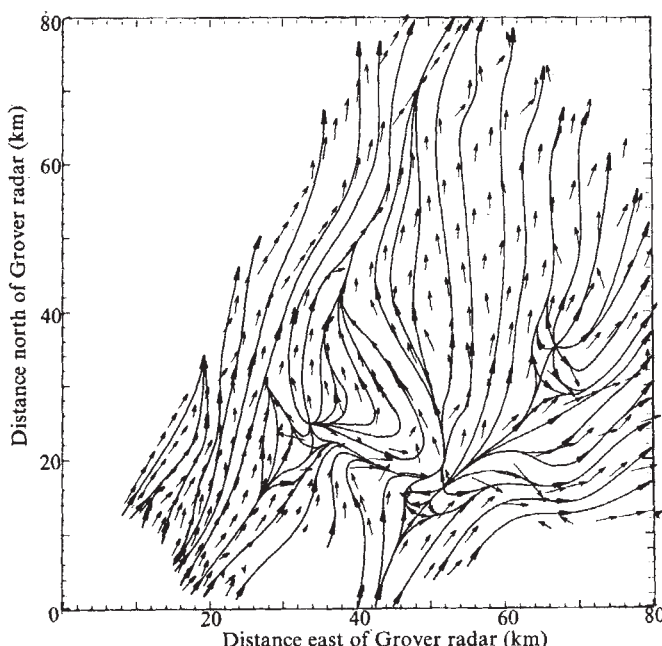


Fig. 3 Reflectivity motion field for data collected on 22 June 1976 at 1627 MDT and 1629 MDT also at 3.3° elevation angle. A subjective streamline analysis is superimposed.

Figure 4 is a reconstructed conical surface at 3.3° tilt centred at the Grover radar. Data for this Doppler analysis were collected from 1625 to 1629 MDT and were obtained from six constant altitude surfaces at 0.5 km height intervals. For clarity, only every third vector from the Doppler data is shown, although the subjective streamline analysis uses all the data.

A comparison of the streamline analyses in Figs 3 and 4 shows reasonable agreement in some locations and considerable difference in others. The agreement at the south-west edge of the storm is quite good in both direction and speed. Large differences exist in regions of rapid change. The main source of difference is caused by the velocity-measuring schemes. Doppler analysis gives instantaneous air motion while TREC determines reflectivity pattern motion. The reflectivity patterns being tracked will often be precipitation generated at a higher altitude. Thus, the pattern may move with the speed and direction of the generating region rather than the velocity appropriate to the area being examined. Figures 3 and 4 show another difference caused by the velocity-measuring schemes. During the two minutes between data scans used in the TREC analysis the whole storm was moving north-northeastwards at approximately 20 m s^{-1} , accounting for some difference in position of the streamline patterns. One of the areas of investigation using the TREC technique is to study the causes and information content of the differences between motion fields detected by the triple-Doppler radar system and TREC. Harris *et al.*¹⁰ give further Doppler analyses from this time period in the 22 June 1976 case which may be compared to the results presented here.

The method clearly may be generalised so that the search for an optimum correlation can be made in three dimensions, utilising data points obtained at a range of tilt angles. Using data from volume scans at different times, vertical velocities might be determined, and data from a single volume scan of the radar where data at adjacent tilt angles are collected nearly simultaneously, enables a streamline pattern to be constructed which would show the slope of the storm at each point.

The procedure outlined above has several possibilities. A first area of development is to optimise the procedure for the phenomena being studied. The 15 by 15 arrays now used are probably not optimal for convective storms. But when the array size is specified in kilometres it will allow various scales of motion to be determined more easily.

A second area of development is to determine vertical motions. We intend to continue using the r, θ, φ coordinate system as far into the processing as possible, waiting until after the full motion field has been determined before transforming to x, y, z coordinates. While we could first interpolate reflectivities into Cartesian coordinates and then try to follow the patterns, we feel that the reflectivity patterns are better conserved in the natural radar coordinate system; attempting to follow these patterns in an x, y, z system would increase the error rate unnecessarily. Additionally, the equation of mass continuity and V_t-Z relationships can be used for this scheme just as they are in dual- and triple-Doppler processing. TREC's ability to measure vertical motions depends upon trackable reflectivity features being present in the storm. For a true steady-state storm, TREC would not be able to detect any motion. In supercells³, inhomogeneities in reflectivity exist which should provide trackable features most of the time. The extent to which TREC can provide vertical velocity measurements will be investigated in the future.

There may be more efficient means of pattern recognition that can be incorporated into the tracking procedure. The simple correlation coefficient procedure seems to work but it does require numerous calculations, thus adding to the expense.

In conclusion, TREC is an objective technique for obtaining quantitative and qualitative information on the three-dimensional motion fields within storms. Arrays of radar reflectivity factor data are correlated from one time or elevation to another. TREC follows reflectivity patterns rather than airflow and these patterns may be moving with the velocities of the generating regions rather than the

velocities of the regions being observed. Thus these results should be interpreted with caution. Nevertheless, this technique seems capable of yielding much more information about internal kinematic structure than has previously been obtained with a single conventional weather radar.

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Global fallout of curium

THE search for ^{242m}Am ($t_{1/2}=152$ yr) in environmental samples has involved detection of its daughter ^{242}Cm ($t_{1/2}=163$ d) (refs 1-3). These samples were contaminated by transuranium nuclides either from a nuclear fuel reprocessing plant or from a thermonuclear test fallout. A logical consequence was then to analyse global fallout-contaminated samples for ^{242}Cm and ^{244}Cm to establish the present distribution and global contamination of our environment by these transuranium elements. We have chosen the lichen *Cladonia alpestris*, which is an excellent bio-indicator for atmospheric fallout and we present the results here. The samples have been stored in the laboratory for 5-16 yr. Thus, ^{242m}Am and ^{242}Cm were in secular radioactive equilibrium. The ^{242}Cm present in the aged samples therefore represents the ^{242m}Am present in the fallout. The results indicate a $^{242m}\text{Am}/^{239+240}\text{Pu}$ activity ratio of about 0.003% in global fallout.

In nuclear power reactors the production of transuranium elements with mass number exceeding 240 can be very substantial. The total activity of ^{241}Am , ^{242}Cm and ^{244}Cm will be a hundred- to a thousandfold greater than that of ^{239}Pu . The highest activity is produced by ^{242}Cm and the activity ratio of $^{242m}\text{Am}/^{242}\text{Cm}$ in waste from nuclear fuel reprocessing is less than 1% (refs 4,5). Thus, it is very difficult to record the activity of ^{242}Cm supported by ^{242m}Am in samples contaminated by discharges from nuclear fuel reprocessing plants.

In nuclear explosions the transuranium nuclides with mass number exceeding 240 are produced in multiple neutron-capture by ^{238}U . The most prominent activity is ^{241}Pu , which decays to ^{241}Am , but small activities of ^{242m}Am ($t_{1/2}, 152$ yr) and ^{242}Am ($t_{1/2}, 16$ h) can also be produced, of which the latter decays rapidly to ^{242}Cm . A transient equilibrium between ^{242}Cm and ^{242m}Am is reached after 3.0 yr. Attempts have been made to show the presence of ^{242m}Am in 12 yr old samples contaminated by fallout by measuring the ^{242}Cm activity³. Observations indicate a $^{242}\text{Cm}/^{239+240}\text{Pu}$ activity ratio of 0.1%, and in one sample a $^{244}\text{Cm}/^{239+240}\text{Pu}$ activity ratio of 0.005% is derived. However, Beasley¹ investigated sediment samples from Bravo Crater in the Bikini Lagoon

Fig. 4 Air motion field for data collected on 22 June 1976 at 1625 MDT to 1629 MDT by a triple-Doppler radar network. The original Doppler data at 0.5-km intervals were reconstructed into a 3.3° tilt conical surface centred at the Grover radar. A subjective streamline analysis is superimposed.

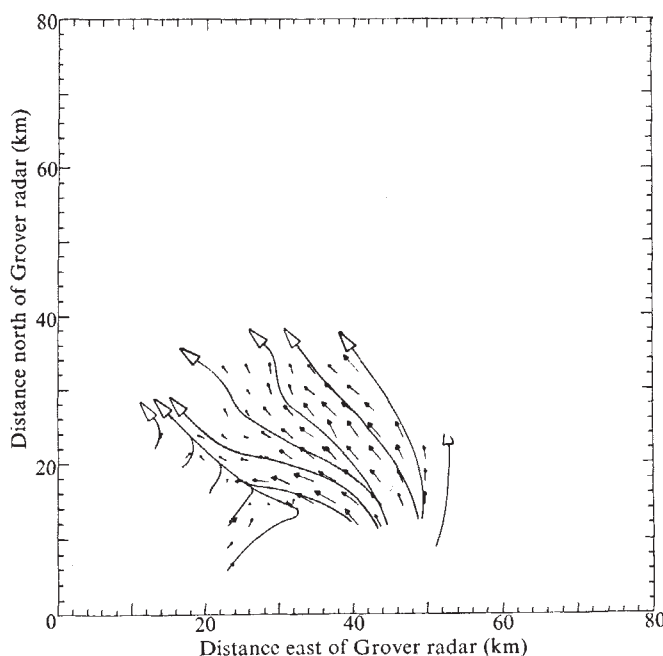


Table 1 Activity concentration (pCi per kg dry weight) of transuranium nuclides in lichen ($\pm$ s.e.m.)

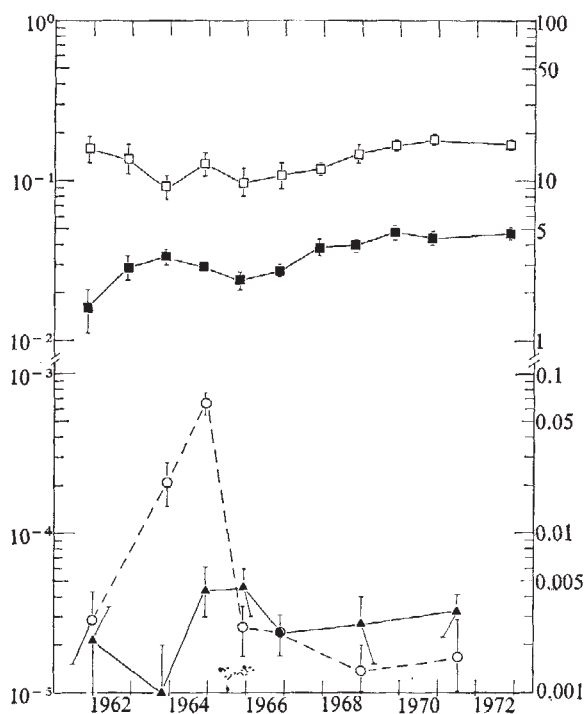
Year	^{238}Pu	$^{239+240}\text{Pu}$	^{241}Pu	^{241}Am	$^{242}\text{Cm}(^{242m}\text{Am})$	^{244}Cm
1961–62	3.3 ± 0.7	140 ± 15	1600 ± 500	22 ± 5	0.003 ± 0.002	0.004 ± 0.002
1963	10.3 ± 1.0	300 ± 45	3800 ± 800	23 ± 5	< 0.003	0.062 ± 0.020
1964	6.6 ± 1.0	230 ± 30	2800 ± 500	34 ± 4	0.010 ± 0.003	0.152 ± 0.020
1965	7.7 ± 0.6	310 ± 30	4300 ± 1100	31 ± 6	0.014 ± 0.004	0.008 ± 0.003
1966	7.5 ± 0.7	250 ± 30	3700 ± 700	26 ± 6	0.006 ± 0.002	0.006 ± 0.002
1968–69	9.7 ± 0.9	220 ± 20	2000 ± 300	35 ± 3	0.006 ± 0.003	< 0.003
1970–72	7.9 ± 0.7	180 ± 20	1600 ± 200	30 ± 3	0.006 ± 0.002	< 0.003

on the Marshall Islands, but found no evidence for the presence of ^{242}Cm or ^{244}Cm .

Lichen samples (*C. alpestris*) have been collected annually since 1961 in central Sweden (62.3°N , 12.4°E) by a standardised technique using a frame over an area of 0.25 m^2 (ref. 6). Lichens very effectively accumulate nuclides from atmospheric fallout and will contain comparatively high activity-concentration levels. Various plutonium isotopes (^{238}Pu , ^{239}Pu , ^{240}Pu and ^{241}Pu) have been previously analysed from these samples^{7,8}. In the present investigation, using samples of 200–500 g of dry lichen, americium and curium were separated by anion-exchange in nitric acid-methanol medium, and extraction with HDEHP (di-2-ethyl hexyl phosphoric acid) according to a procedure described elsewhere⁹. The actinide isotopes were subsequently electroplated onto stainless steel disks. Each sample was counted with surface-barrier detectors for 20,000 min or until 100,000 counts were recorded under the ^{241}Am peak.

The samples were analysed for curium during 1977 and the results are shown in Table 1, where data from previously analysed ^{238}Pu , $^{239+240}\text{Pu}$, ^{241}Pu and ^{241}Am are also included. All results are corrected to the date of collection, then taking any ^{242}Cm -activity recorded as supported by ^{242m}Am .

Fig. 1 The ratio of the activity concentrations of ^{242}Cm (^{242m}Am) ($\blacktriangle$), ^{244}Cm ($\circ$), ^{238}Pu ($\blacksquare$) and ^{241}Am ($\square$) to $^{239+240}\text{Pu}$ in lichen samples collected during 1961–72. The ratio is given in % on the right ordinate.



These data indicate an ^{242m}Am content (with respect to ^{241}Am) of approximately 0.02–0.04%, which is a considerably lower value (by a factor of 10) than that found by Bowen and Livingston³. This indicates that the data reported by them were not typical for global fallout. The ratio of the activity concentrations of ^{242}Cm , ^{244}Cm , ^{238}Pu and ^{241}Am to $^{239+240}\text{Pu}$ in the lichen carpet are indicated in Fig. 1 for the period 1961–72. The average $^{242}\text{Cm}/^{239+240}\text{Pu}$ activity ratio is observed as approximately $(0.003 \pm 0.001)\%$ during the period 1961–72, which is much lower (by a factor of about 20) than the value reported by Bowen and Livingston³. The latter group, however, analysed intact samples from a particular explosion very different from global fallout-contaminated samples, whereas the present values are influenced by the different varying biophysical behaviour of americium and curium in the environment. The highest $^{244}\text{Cm}/^{239+240}\text{Pu}$ activity ratio of 0.07%, which we recorded in 1964, is surprisingly high. Beasley¹ found no ^{244}Cm in samples from Bravo Crater, and Bowen and Livingston³ recorded a $^{244}\text{Cm}/^{239+240}\text{Pu}$ activity ratio of 0.005% in only one of the samples. A few years later the $^{244}\text{Cm}/^{239+240}\text{Pu}$ activity ratio was fairly constant at an average value of 0.002%, which is in good agreement with observations by Bowen and Livingston³. The rapid decrease of the $^{244}\text{Cm}/^{239+240}\text{Pu}$ activity ratio after 1963–64 indicates a shorter biological mean residence time for curium than for plutonium in the lichen carpet immediately after deposition. By using the biological mean residence time for plutonium, which has been previously determined to be 6 yr (refs 7,8), the biological mean residence time for curium was estimated to ~ 150 d by using the gradient of the $^{244}\text{Cm}/^{239+240}\text{Pu}$ ratio over 1964–66 in Fig. 1.

The $^{238}\text{Pu}/^{239+240}\text{Pu}$ activity ratio in global fallout from nuclear trial detonations is approximately 0.026. The $^{242}\text{Cm}/^{239+240}\text{Pu}$ activity ratio in fresh debris (after first decay of ^{242}Am) is estimated to be of the order of 0.10. However, only 1.5% of the ^{238}Pu activity can be explained by the decay of ^{242}Cm . The reaction $^{238}\text{Pu} (n, 2n) ^{237}\text{Pu}$ is probably more important. In nuclear reactors the decay of ^{242}Cm is more important for the production of ^{238}Pu , with the reaction $^{237}\text{Np} (n, \gamma) ^{238}\text{Pu}$.

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Possible causes of ice sheet thinning in the Mizuho Plateau

To measure the horizontal and vertical movements and strain rates of the ice sheet surface in the Mizuho Plateau, East Antarctica, the Japanese Antarctic Research Expedition carried out the first triangulation survey on the network in November–December 1969 and a resurvey in December 1973–January 1974 along a route shown in Fig. 1 (see ref. 1). The surface slope¹ and accumulation rate² along the route were also measured. The route starts from point B fixed on rock in the Yamato Mountains and it traverses flow lines most of which are converging into the Shirase Glacier. The route is almost parallel to the equal elevation contours of the ice sheet surface except near points B and C. We report here the thinning of the ice sheet and discuss how it was caused.

Using the vertical movement, Naruse³ calculated the rate of change, $\dot{h}$, of the ice thickness, h , by excluding the effect of the firm densification, as shown in Fig. 2. The possible error of the vertical movement resulting from refraction in the atmosphere and curvature of the Earth was counterbalanced because the vertical angles were measured twice from two positions of the triangulation chain in the opposite direction and the angles were averaged. Consequently, the mean square error of the vertical movement is 18 cm yr^{-1} at point A and 31 cm yr^{-1} at point C (ref. 3). The mean square error of $\dot{h}$ is approximately equal to that of the vertical movement. Figure 2 indicates that $\dot{h} \sim 0$ between points A and B but $\dot{h} \sim -70 \text{ cm yr}^{-1}$ between points A and C. As shown in Fig. 3, the horizontal surface velocity, u_s , also changes drastically at point A.

The average accumulation rate along the route is $\sim 7 \text{ cm yr}^{-1}$ and the maximum accumulation rate is $\sim 30 \text{ cm yr}^{-1}$ (ref. 2). Even in the coastal area where cyclones can occur and the amount of precipitation is large the accumulation rate rarely exceeds 50 cm yr^{-1} (see ref. 4). Therefore, the thinning rate is too large to be compensated for by the present accumulation rate observed in the Mizuho Plateau, and temporal variations of the accumulation rate cannot account for such a large thinning rate.

Fig. 1 Map of the Mizuho Plateau. The survey route is represented by a thick solid line. Equal elevation contours are represented by thin lines.

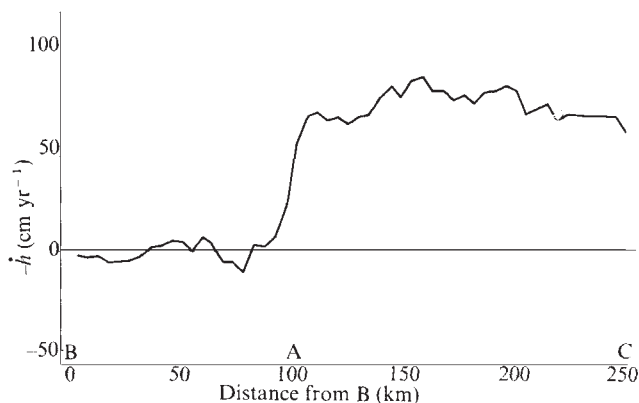
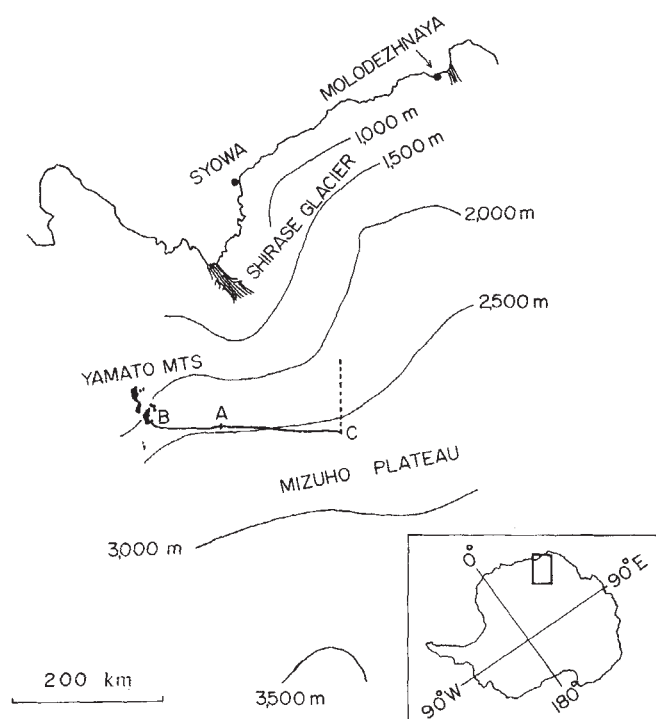


Fig. 2 Rate of change, $\dot{h}$, of the ice thickness, h , along the survey route.

Thomas⁵ proposed that the ice at Byrd Station, West Antarctica, is thinning by $\sim 40 \text{ cm yr}^{-1}$ and it commenced $\sim 2,000 \text{ yr}$ ago. At present, we have no evidence of when the thinning commenced in the Mizuho Plateau, but as the thinning rate is $\sim 70 \text{ cm yr}^{-1}$ the thinning might have occurred $\sim 1,000 \text{ yr}$ ago. Therefore, it seems that the thinning of the ice sheet is a general phenomenon over the Antarctic Continent except for the central cold part and that it started a few thousand years ago.

Now we consider possible causes of the thinning. If we assume that the strain rate is independent of depth, and the freezing or melting rate at the base is neglected, the equation of mass continuity along the flow line is given by⁶

$$\dot{h} = \dot{A} + u_s \alpha_s - u_b \alpha_b - (\dot{\epsilon}_x + \dot{\epsilon}_y)h \quad (1)$$

where $\dot{A}$ is the accumulation rate, u_b is the horizontal velocity at the base, α_s and α_b are the slope at the surface and the base and $\dot{\epsilon}_x$ and $\dot{\epsilon}_y$ are the strain rates parallel and perpendicular to the flow direction, respectively. Substituting the values of $\dot{h}$, $\dot{A}$, u_s , α_s , h (Fig. 3) and $\dot{\epsilon}_x + \dot{\epsilon}_y$ (Fig. 4), we obtain $u_b \alpha_b$, as shown in Fig. 4.

The flow line on the route generally converges slightly. However, when $\dot{\epsilon}_x + \dot{\epsilon}_y$ is extraordinarily large or small the convergence or divergence of the flow lines is large; we should not use equation (1) at these points. Excluding the large strain rate, between points A and B, $u_b \alpha_b \sim 0$, so $u_b \sim 0$. Between points A and C, the average values of $\dot{A}$, $u_s \alpha_s$, $(\dot{\epsilon}_x + \dot{\epsilon}_y)h$ and $\dot{h}$ are 9, 7, 31 and -70 cm yr^{-1} , respectively. Substituting these values into equation (1), we obtain $u_b \alpha_b \sim 55 \text{ cm yr}^{-1}$. This suggests that u_b is significantly large and the thinning of the ice sheet may be predominantly caused by the basal sliding because:

$$u_b \alpha_b \gg \dot{A} + u_s \alpha_s - (\dot{\epsilon}_x + \dot{\epsilon}_y)h$$

Using the bedrock topography obtained by the radio echo sounding along the dashed line in Fig. 1 (ref. 7), α_b is estimated to be $\sim 4.5 \times 10^{-2}$. As $u_b \alpha_b \sim 45 \text{ cm yr}^{-1}$ at point C, $u_b \sim 10 \text{ m yr}^{-1}$.

The basal shear stress is given by $\tau = \rho g h \alpha_s$, where ρ is the density of ice and g is the gravitational acceleration. The calculated basal shear stress along the route is shown in Fig. 4. There is a low τ zone ($\tau \sim 0.7 \text{ bar}$) around 40 km west of point A and $\tau \sim 1.2$ or 1.3 bar near point A. The mean value of τ between points A and C is $\sim 1.0 \text{ bar}$, so the increase of u_b observed may be due to increased internal deformation within the ice mass. However, the basal sliding might be taking place between points A and C and also contribute to the large surface velocity observed there. One reason is that α_s around point A is large only at the route and average τ along the flow line around point A is smaller than τ adopted here. The other reason is that the surface velocity, u_p , due to internal deformation, is calculated using an equation of u_p for ice sheets obtained by Hughes⁸ under an assumption that

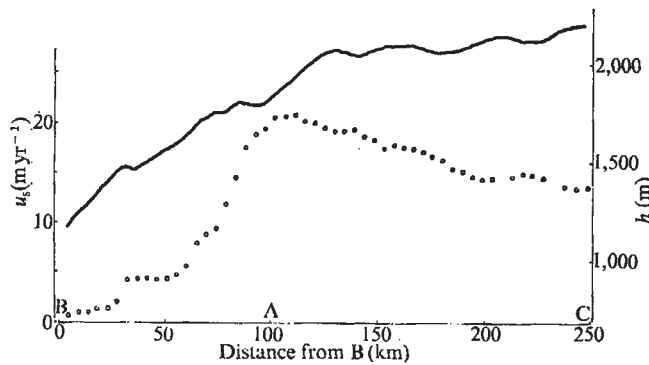


Fig. 3 The rate of change of the horizontal surface velocity u_s (○), and h (solid line) along the survey route.

the base of the ice sheets is at the melting point. As a result, at the low τ zone u_p is $\sim 5 \text{ m yr}^{-1}$ for $\alpha_s = 5.5 \times 10^{-3}$ and $h = 1,500 \text{ m}$, and between points A and C u_p is $\sim 11 \text{ m yr}^{-1}$ for $\alpha_s = 5.0 \times 10^{-3}$, the average value between points A and C, and $h = 2,100 \text{ m}$. Overestimation of u_p at the low τ zone where u_s is 4 m yr^{-1} indicates that the base is not at the melting point but is frozen. The result that $u_s > u_p$ between points A and C may suggest that the basal sliding contributes to the large u_s and the base is at the melting point.

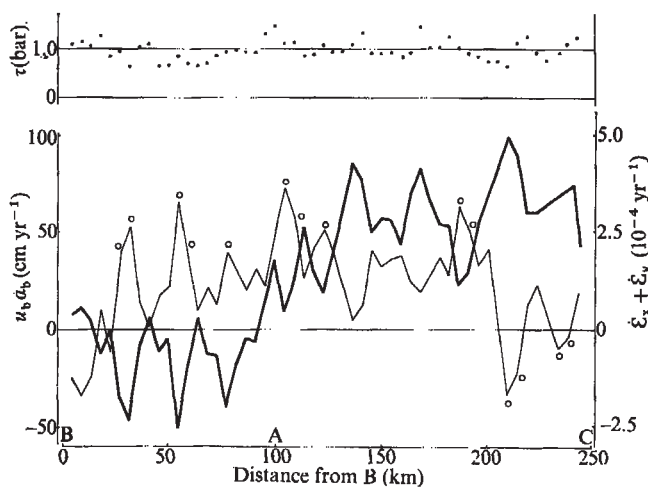


Fig. 4 The rate of change of $u_s \alpha_b$ (heavy line), $\epsilon_x + \epsilon_y$ (fine line) and τ (●) along the survey route. ϵ_x and ϵ_y are calculated from principal strain rates³. Extraordinarily large or small strain rates are indicated by open circles.

If the basal ice is at the melting point and the basal sliding is occurring between points A and C, at least in the northern part of the Shirase Glacier drainage basin from the route the base is wet. This may be a reason for the rapid motion of the Shirase Glacier, $u_s \sim 2.2 \text{ km yr}^{-1}$, as if it were surging.

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Age-length dependence of inclined seismic zones

HEAT flow, and hence lithospheric temperature, in the South Atlantic, North Pacific and East-Central Pacific has been shown^{1,2} to decrease systematically with increasing age of the sea floor. As the oceanic lithosphere is consumed at a subducting plate margin, the limiting depth of earthquakes associated with the downgoing plate is ultimately determined by the temperature at which the lithosphere becomes too hot for brittle deformation to occur³. Isacks et al.⁴ have demonstrated that a crude correlation exists between the length of inclined seismic zones and the rate of consumption of lithosphere. However, Hyndman and Riddiough⁵ have commented that aseismicity may well result in regions where young lithosphere, at elevated temperatures, is being subducted. Here, we evaluate the latter idea and further, on the basis of a study of South American seismicity, we propose that there is a relationship between the length of inclined seismic zones and the age of the sea floor being subducted.

To test this hypothesis a plate interaction must be studied where other parameters that may affect plate seismicity are minimal. This means that only regions with similar consumption rates can be compared⁴. Furthermore, a consuming margin over which there is a large variation in the age of plate being consumed is desirable. Hence, the Nazca-South American plate convergence was chosen for study. To eliminate the perturbing seismic effects that may accompany plate segmentation interactions along the curved portion of the Peru-Chile trench⁶, only the seismicity associated with the linear portion of the convergence (26°S to 44°S) was considered.

The angular velocity of the Nazca-South American plate convergence is $0.99^\circ \pm 0.11^\circ \text{ Myr}^{-1}$ about an axis through a pole at 51.9°N, 91.4°W (ref. 7) thus the average azimuth

Table 1 Age of lithosphere around the Nazca-South American plate

Section	T_p (Myr)*	Age gradient cm yr^{-1}	T_B (Myr)†	T_C (Myr)‡
26°–28°S	53	0.17	65	59
28°–30°S	53	0.17	67	60
30°–32°S	47	0.17	59	53
32°–34°S	43	0.17	54	48
34°–36°S	39	0.17	48	43
36°–38°S	35	0.17	41	38
38°–40°S	32	0.24	39	36
40°–42°S	22	0.24	25	24
42°–44°S	15	0.24	18	17

*Present age of lithosphere entering trench.

†Present age of lithosphere at lower termination of seismic zone.

‡ $T_C = T_B - (\text{length of zone}/\text{convergence rate})$.

of convergence is N85°E and, because the region studied is astride the equator of convergence, the linear rate of consumption deviates by less than 2% from its maximum value (11 cm yr^{-1}) at 38°S. Therefore, variations in length of inclined seismic zone due to differences in convergence rate⁴ are negligible.

NOAA hypocentre data for the years 1963–73 were used as the basis for the generation of vertical two dimensional sections. When all earthquakes of magnitude greater than 4 are used, optimum definition of the inclined seismic zone occurs with sections 2° wide. Thus nine sections were plotted between 26°S and 44°S.

The linear portion of the Peru-Chile trench trends N5°E and thereby constrains⁶ the subduction direction to N95°E. However, because the convergence direction is N85°E,

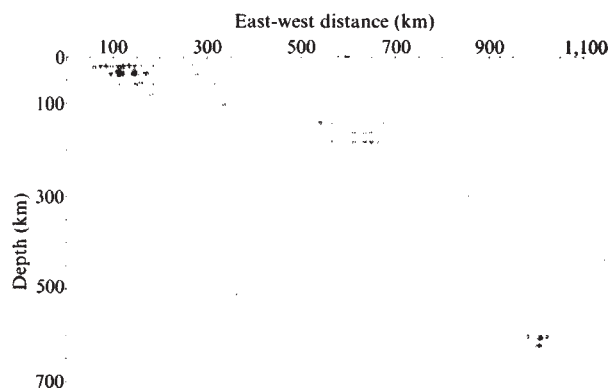


Fig. 1 East-west cross section showing hypocentres between 28° and 30°S lat.

sections were generated in a compromise east-west orientation. Figure 1 showing hypocentres between latitudes 28°S and 30°S is typical of the nine sections drawn.

The corrected age (T_c) of the lithosphere, listed in Table 1, represents the age that that part of the plate currently being resorbed at the lower end of the inclined seismic zone had when it entered the subduction zone. First the age of oceanic lithosphere currently at the trench (T_p) was estimated by interpolation between known magnetic anomalies⁸⁻¹¹. Then, using the sea floor age gradient characteristic of that part of the Nazca plate about to be subducted, the age of the lithosphere currently being resorbed (T_E), was calculated. Finally, using the present consumption rate (11 cm yr⁻¹), a corrected age (T_c) was determined for each section. The absolute ages calculated in this manner may well be in error by as much as ± 5 Myr, however, if the age gradient in the subducted lithosphere is uniform and, because the age of the crust increases monotonically northwards from the Chile Ridge, the uncertainties in relative ages between sections will be much smaller.

Figure 1 shows that a problem arises in determining the lower limit of the inclined seismic zone. We have defined the length of the inclined seismic zone to be the distance measured along the locus of maximum hypocentre density, from its point of intersection with the surface (near the Peru-Chile trench) to the point at which the hypocentre density falls to less than 25% of the maximum density occurring in that section. The hypocentre density distribution for each section was calculated on the basis of the

number of hypocentres appearing in a 75 km square area. In Fig. 2 hypocentre density-down dip distance profiles, normalised to the maximum density value occurring in the section, are plotted against corrected age for each 2° segment. Because of the controversy surrounding the mechanism of deep focus earthquakes and the continuity of the downgoing slab^{12,13}, these earthquakes, although plotted, are treated separately. A smooth curve (Fig. 2) has been drawn to delimit the lower end of the inclined seismic zone, using the 25% density cutoff as a guide.

We suggest that the relationship revealed by the curve between the length of the inclined seismic zone and age arises because older oceanic lithosphere, being cooler at the onset of subduction, survives longer seismically before being thermally resorbed. The resorption curve is approximately exponential and has a time-constant of 24 Myr. The oceanic lithosphere cooling time-constant determined from heat flow studies in the Pacific^{1,2} is in the vicinity of 35 Myr. The difference in the cooling and resorption time-constants may possibly be explained by the higher boundary temperatures and frictional heating of the plate being resorbed. The deep focus earthquakes do not seem to fall on the same trend as the shallow and intermediate depth earthquakes, which may reflect lack of continuity of the inclined seismic zone or a different earthquake mechanism.

Future studies using more precisely determined hypocentre locations and higher hypocentre densities, may be better for revealing the nature of the curve and for enabling the thermal and elastic properties of the downgoing slab to be determined.

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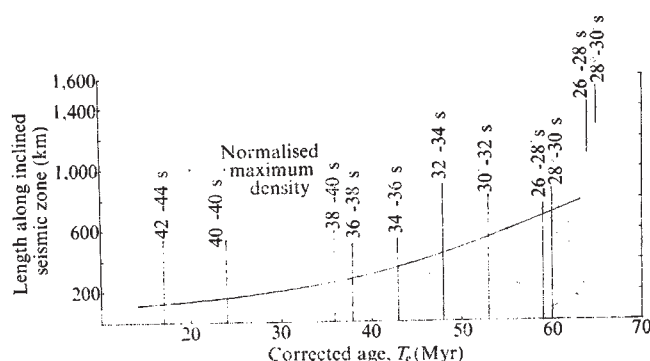
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Fig. 2 Plot of length of inclined seismic zone under South America against the age that the lithosphere had at the onset of subduction.



Behaviour and speciation of dissolved selenium in estuarine waters

THE behaviour of chemical species in estuaries¹ is important for several reasons. The extent of any modification of the riverborne flux of dissolved materials to the open sea, as a result of interactions during the mixing of fresh and saline waters, is of fundamental geochemical significance. Knowledge of the pathways of element transference in estuaries is needed to predict effects of waste inputs and other modifications of estuarine conditions. It is also in the estuarine zone that the limitations of the determination and modelling of chemical speciation become most acute, because of the

wide range of major compositional variables and the importance of kinetic factors. Among the major problems is the uncertainty as to the true redox potentials of natural waters and the factors which can lead to the presence of thermodynamically unstable oxidation states. Recent work^{2,3} on selenium in seawater has indicated that the redox speciation of this element shows some differences from that predicted for an equilibrium system. We give here results of measurements of selenium in some river and estuarine waters of the Solent area. Available Se(IV) was detected in chalk streams but always accounted for less than 10% of the total dissolved selenium. Dissolved forms of selenium behaved essentially conservatively during estuarine mixing of chalk stream inputs.

The Solent, in mid-southern England, receives inputs from rivers which drain geologically contrasting areas. Samples were collected from the rivers listed in Table 1 in late July 1977, and in early August surveys were made, on the estuarine part of the Test and its continuation, Southampton Water, and on the Beaulieu Estuary, in which samples were collected over a range of salinities. The River Beaulieu drains marshes and heathlands of the New Forest and has much higher concentrations of dissolved organic matter, iron and manganese^{4,5} than the other listed rivers which mainly drain chalk strata.

Samples were collected and stored in polypropylene vessels, filtered through membrane filters (0.45 μm average pore diameter), and analysed within at most 12 h of collection. Salinity was measured on sub-samples (which were stored in glass bottles) using an inductively coupled salinometer. Available Se(IV) was measured by formation of the complex with 4-nitro-*o*-phenylenediamine at pH 1 in the presence of EDTA followed by its extraction into toluene and determination by gas-liquid chromatography using electron-capture detection. The method is a development⁶ of the procedures described by Shimoishi⁷, and Gosink and Reynolds⁸. Total dissolved selenium was determined by the application of the same procedure to aliquots which had been photo-oxidised by UV irradiation using a 1,000-W medium pressure mercury arc lamp, the solution being buffered by addition of 2 ml of 0.1M sodium tetraborate solution to 100 ml of sample. In these conditions reproducible disproportionation of selenium between the oxidation states (IV) and (VI) occurs with a yield of Se(IV) of $74 \pm 3\%$, which is independent of both salinity and the initial oxidation state of selenium⁶.

The redox reactions which occur in natural waters during analytical photo-oxidation are not well understood. Partial reduction of nitrate to nitrite during irradiation of seawater over 3 h was noted by Armstrong *et al.*⁹ In a study of the photochemistry of nitrate in aqueous solution, Shuali *et al.*¹⁰ reported the formation of nitrite, with evolution of an equivalent amount of oxygen; the extent of reduction was dependent on irradiation time and varied markedly with pH, with maximum conversion above pH 10. A marked effect of pH on the yield of selenite was observed in the present work.

Table 1 Concentrations of selenium and calcium in river waters

River	Dissolved selenium (ng l ⁻¹)		Dissolved calcium (mg l ⁻¹)
	Total	Se(IV)	
Test*	369	18	83
	376	21	82
Itchen	250	18	85
Hamble	390	31	83
Meon	278	20	84
Beaulieu*	85	<2	28
	73	<2	10
	76	<2	13

*Results from different sampling points are given.

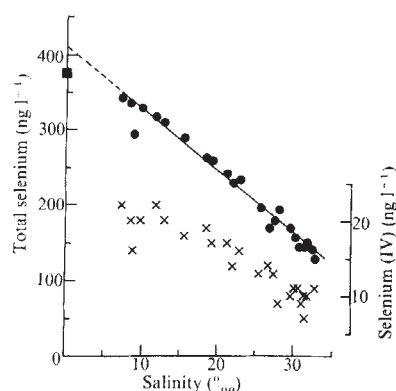


Fig. 1 Relationships between salinity and the concentrations of total dissolved selenium (●) and dissolved Se(IV)(×) in Southampton Water and the estuarine part of the River Test. The regression of total dissolved selenium on salinity, with its extrapolation to zero salinity, is shown. The concentration of total dissolved selenium in River Test water sampled before the estuarine survey is also given (■).

The difference between the total dissolved selenium and the available Se(IV) represents an operationally defined fraction which could include several forms of selenium, for example, organically associated forms. From consideration of the solution chemistry of the element and its behaviour during photo-oxidation⁶, Se(VI), as the unprotonated selenate ion, is the most probable species in this fraction. This conclusion contradicts the view, presented, for example, by Leutwein¹¹, that selenate is unstable in normal redox conditions. Sillén's calculations¹² showing that selenate is strongly dominant for an equilibrium oxalic aqueous system at a pH of 8.1 and pE of 12.5, seem, however, entirely sound, and from the same data it is apparent that selenate remains dominant even at the much lower pE of 8.5 postulated by Breck¹³. These thermodynamic considerations are reinforced by other observations^{2,3} of the presence of a species in natural waters which behaves like Se(VI).

The results for river waters (Table 1) show that the concentrations of total dissolved selenium in the chalk streams are similar, with an average value (330 ng l^{-1}) four times higher than that in the more acidic River Beaulieu. Selenium(IV) accounted for between 5 and 8% of the total selenium in the chalk streams. In the River Beaulieu this species was below the limit of detection and could not account for more than some 2.5% of the total. The difference in speciation between the systems is thus small but seems significant. It might reflect differences in the sources of selenium in the drainage areas but it is also notable that the River Beaulieu is a system in which there is substantial removal of iron from the dissolved fraction⁴ and it is possible that particulate hydrous iron oxide is responsible for scavenging Se(IV), in accordance with its known geochemical behaviour¹¹.

Figure 1 shows that the concentrations of total dissolved selenium in Southampton Water and the estuarine sector of the River Test decrease in a closely conservative manner over the salinity range 7–34‰ and the regression of concentration on salinity predicts a river water concentration (410 ng l^{-1}) which is similar to that observed for the River Test before the survey (Table 1). The relationship of Se(IV) to salinity, despite a greater scatter probably attributable to decreased analytical precision at the low concentrations concerned, also indicates conservative behaviour; regression analysis using the data for this species predicts a river water concentration of 25 ng l^{-1} , which again agrees satisfactorily with the measured values.

Concentrations of total dissolved selenium in the Beaulieu Estuary showed more complex variation with salinity. Concentrations at the lowest salinities examined, about 13‰, were $\sim 130 \text{ ng l}^{-1}$ and were thus higher not only than in the seawater mixing in the estuary (containing 79 ng l^{-1} at $S=34\text{‰}$ but also than in the river water sampled before the survey (Table 1). Over the salinity range 13–34‰ the concentrations decreased in a broadly linear manner but with considerable scatter. This pattern of distribution might suggest the desorption of some selenium from particulate material in the earlier stages of estuarine mixing but the concentration changes seem too large to be fully explained in this way. The Beaulieu system is subject to widely fluctuating conditions of flow which may complicate interpretation in terms of a mixing series with fixed end members. More detailed investigations are needed to assess whether in this chemically interactive system selenium shows differences from its essentially conservative behaviour during estuarine mixing of chalk-stream inputs. A nonlinear relationship between total selenium and salinity has been reported by Sugimura *et al.*² for coastal waters receiving freshwater inputs with a lower concentration of selenium than that in the seawater.

Concentrations of selenium in river waters, excluding those draining seleniferous regions, have been reported^{2,3,14–16} to range over 10–350 ng l^{-1} . Turekian¹⁷ in a compilation of 'first estimates' of the trace element concentrations of streams, gives a value for selenium of 200 ng l^{-1} . With sparse data and values for various rivers showing a range of concentration covering between one and two orders of magnitude, estimates of global averages are necessarily very approximate, even more so than for the major constituents. If the relatively high values for selenium and rather constant ratios of selenium to calcium shown by the chalk streams listed in Table 1 were confirmed for other regions, a calculation using a mean ratio and a global average concentration for calcium in river water might give an improved estimate. Such a calculation on the basis of the present data and Livingstone's value for calcium in the global average river water supply¹⁸ would suggest a mean concentration of selenium in the global river input of 60 ng l^{-1} .

The present findings indicate that thermodynamically unstable Se(IV) is present in some river waters as a minor fraction (less than 10%) of the total dissolved selenium. Hiraki *et al.* report¹³ that Se(IV) accounted for 2 to 16% of the total selenium in samples from two rivers in eastern central Japan, whereas Miyake *et al.*¹⁶ found it to comprise 75% of the dissolved selenium in the Kuji-gawa River. Several investigations^{2,3,6} show that Se(IV) forms a substantial fraction of the dissolved selenium in some oceanic waters, particularly deep waters. Most of the information on river waters suggests that the riverborne supply of Se(IV) is inadequate to explain this phenomenon, which probably arises through the cycling of the element in the marine biosphere.

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Biochemical evidence of a former link between Australia and the Mascarene Islands

THE land masses destined to become Australia, Madagascar and the associated Mascarene Islands are thought to have separated from the old southern continent of Gondwanaland after the establishment of an angiosperm flora¹. Similarities in the present floras of the areas, as exemplified by *Cohnia* and *Cossinia* which occur in Australia and New Caledonia but have their most westerly habitats in the Mascarene Islands, are consistent with this belief². The legume genus *Acacia* which is widely represented in America, Africa, Asia, Australia and many oceanic islands is thought to have originated in what is now northern South America and to have spread through Gondwanaland³. A survey of non-protein amino acids in the seeds of 123 species of *Acacia* has shown that the genus is divisible into four discrete biochemically defined sub-genera⁴. All 67 species from Australia which were analysed showed a single characteristic amino acid pattern. This Australian pattern was found in only one non-Australian species, *A. heterophylla* (Lam.) Willd. a native of the Mascarene Islands and one of the few phyllodinous species found outside Australia. In this communication we suggest that the seed chemistry of *A. heterophylla* and of the Australian species is the seed chemistry which characterised the original *Acacia* species of Gondwanaland.

In spite of striking morphological variability between adult plants such as the replacement of leaves by phyllodes in the Phyllodineae, the seeds of all species of *Acacia* from Australia and of *A. heterophylla* were found to contain the same four non-protein amino acids, albizziine, S-carboxyethylcysteine, S-carboxyisopropylcysteine and α -amino- β -acetylaminopropionic acid. Most nearly related to these species in terms of amino acid chemistry was a second group of species whose seeds contained the same four non-protein amino acids together with α , β -diaminopropionic acid and the neurotoxin α -amino- β -oxalylaminopropionic acid. The biochemical difference between these two groups although readily recognisable, probably represents a minor genetic difference, possibly of one or a few genes. Species containing the neurotoxin are found in both Africa and Asia. The seeds of two island acacias, *A. kauaiensis* Hbd. a phyllodinous species from the Hawaiian islands and *A. confusa* Merr. from Formosa also contained the oxalyl amino acid, suggesting that they are probably of Asian rather than Australian origin.

The biochemical differences between the remaining groups and the first and second groups seem to be more fundamental. The species of the third group which are found variously in Africa and Asia belong to the series Gummiferae of Bentham's classification⁵ and to the sub-genus *Acacia* of Vassal's classification³. They are characterised by the presence of high concentrations of acetyldjenkolic acid in their seeds. Species of the fourth

group which occur in both Africa and America belong to the subgenus *Aculeiferum* section *Monacantha* of Vassal's classification and form part of the series *Vulgares* of Bentham's classification. Unlike the species of the first three groups they do not accumulate high concentrations of non-protein amino acids in their seeds.

The occurrence of species of one of the biochemically defined sub-genera of *Acacia* in both Australia and the Mascarene Islands suggests that these two habitats once formed part of the same land mass. The restriction of the sub-genus to the two areas also suggests, however, that geographical isolation spared the Australian species and *A. heterophylla* from competition with those species of differing seed chemistry which have become dominant in Africa, Asia and America. Although the Australian pattern does not seem to have survived outside that continent and the Mascarene Islands, the modified Australian pattern which includes the neurotoxin α -amino- β -oxalylaminopropionic acid is found in several Asiatic and African species. This modified pattern probably arose as the result of minor changes in the genome of the Australian type. The presence of an additional seed constituent which is toxic or deterrent to mammals^{6,7}, birds⁸ and insects^{8,9} may indeed have discouraged potential predators and provided the selective advantage which has led to the establishment of species with the modified pattern in areas other than Australia.

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Concealed inefficiency of late-night study

PRESSURES of work often force people to stay up late, attempting to commit material to memory during the early hours of the morning. 'Burning the midnight oil' in this way often seems reasonably efficient at the time, despite the fact that a wide range of physiological and psychological circadian (24 h) rhythms are then at their lowest point. However, recent research would predict that although people's immediate memory for the material studied might be reasonably good, much more will be forgotten with time than if the learning had taken place at a more 'normal' time of day. This prediction was tested and verified on a group of part-time night workers. An 'in service' training film was shown at either 2030 h or 0400 h. When tested immediately after seeing it, memory for the film was, if anything, slightly better at 0400 h than at 2030 h. However, the group that was shown the film at 0400 h forgot more than twice as much over a 28-d period as the group shown it at 2030 h. Thus, although studying late at

night might seem to be efficient at the time, long-term retention of the material will be rather poor.

Ebbinghaus was well aware of the influence of time of day on memory and controlled for these effects in his pioneering work at the end of the nineteenth century. Early studies^{1,2} of time of day effects were concerned with relatively short-term retention which was found to be worse in the afternoon than in the morning. These results were interpreted in terms of 'mental fatigue', which was thought to build up during the waking day. In contrast, later studies^{3,4} attributed this afternoon inferiority to a build-up in arousal. Arousal is considered to increase over the waking day, peaking in the evening and showing a trough in the early hours of the morning, paralleling the circadian rhythm of body temperature⁵. Although high arousal improves performance on many simple processing tasks⁵, there is some indirect evidence that it might be detrimental for short-term retention.

An arousal interpretation such as this would also predict that rather more would be forgotten following presentation when body temperature is low. This has been confirmed in a memory study⁶ in schoolchildren for a story read to them at either 0900 h or 1500 h. Memory for the story was tested either immediately after hearing it, or after a week's delay. Immediate recall was better in the morning, but delayed recall was better following afternoon presentation.

An opportunity to test this prediction at more extreme times of day occurred as part of a larger study of circadian rhythms and their adjustment in night-working nurses⁷. The 26 part-time nurses studied (27 actually took part, but one was omitted because of her previous knowledge of some of the material presented in the film) exhibited normal, day-orientated circadian rhythms^{8,9}, and were thus representative of day-workers who occasionally stay up late. A training film on nursing procedures during radium therapy was shown to approximately half of the nurses at 2030 h and half at 0400 h. The shift lasted from 2045 to 0745 h. Their memory for the information presented in the film was tested both immediately and after a 28-d delay. Two parallel questionnaires were used in a counter-balanced order. Each questionnaire consisted of 15 questions requiring single-word or short-phrase answers and 5 multiple-choice questions. In the delayed test, half the nurses were tested at the same time of day as they had originally seen the film, and half at the other time of day. This allowed us to examine the effects of time of testing per se and mis-match between times of testing and presentation, in the delayed condition.

Just before the film, oral temperatures were recorded; they averaged 36.6 °C for the 2030-h group and 36.4 °C for the 0400-h group (s.e.m. 0.1 °C in each case). In the immediate memory test, the 2030-h group answered 55.0% of the questions correctly, and the 0400-h group scored 59.2%. This slight superiority of the 0400-h group in immediate memory was not statistically significant ($t < 1$). As a measure of the amount forgotten over the 28-d period, each nurse's delayed memory score was subtracted from her immediate memory score and the difference expressed as a percentage of the immediate score. Over the 28-d period, the nurses shown the film at 0400 h forgot over twice as much as those shown it at 2030 h (39.5% against 15.5%). This difference in amount forgotten was statistically reliable ($t = 3.52$, d.f. = 23, $P < 0.01$).

Analyses of the delayed memory scores indicated that there were no reliable differences either between testing at 2030 h and testing at 0400 h or between matched and mismatched testing and presentation times. Both of these negative results agree with those of the schoolchildren study mentioned above⁶. They indicate that the amount remembered is more dependent on the time of day at which the material is learned than that at which it is tested.

As staying up late at night is usually associated with some degree of sleep deprivation, the effects described here are probably not solely attributable to circadian rhythms. However, the similarity between these results and those of the schoolchildren study⁶ do suggest that circadian rhythms had an important role in producing them. Whatever their cause, these effects undoubtedly indicate that late-night studying is much less efficient than one's immediate impressions might imply. Much more will be forgotten later on than if the studying had taken place at a more normal time of day.

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Memory in monkeys severely impaired by combined but not by separate removal of amygdala and hippocampus

THE profound anterograde amnesia that has been attributed in the clinical literature to damage of the hippocampal system^{1,2} has not been observed in animals with such damage. Hippocampal-system lesions in animals do markedly impair some forms of spatial memory^{3,4}, but the effects on other forms of memory have generally seemed minor^{5,6} compared with the dramatic disorder described in man^{7,8}. This discrepancy between the clinical and animal literature could indicate a true evolutionary shift in the functions of the hippocampus^{9,10}, or, at the other extreme, it could simply reflect the use of incommensurate measures across species^{11,12}. (Strong support for this second interpretation has been provided by Gaffan¹³.) A third possibility, however, is that the discrepancy points to inaccurate localisation of the neuropathology in man that is responsible for the profound amnesia. Support for this last alternative comes from new evidence in monkeys indicating that a striking impairment in visual memory can be produced by the combined ablation of the hippocampal formation and the amygdaloid body, but not by ablation of either of these structures alone.

Twelve monkeys (*Macaca mulatta*) weighing from 3.5 to 5 kg were trained preoperatively in a Wisconsin general testing apparatus to perform one-trial object recognition¹⁴ for peanut rewards. The procedure involved presentation of a baited object over the central well of a three-well tray, followed 10 s later by re-presentation of that object (now negative) together with a new object (positive) over the lateral wells. Twenty such trials separated by 30 s intervals were given daily, each trial with a new pair of objects. The successive pairs were drawn randomly from a collection of several hundred unmounted junk objects of diverse sizes, colours, shapes and textures. The testing procedure exploits

the monkey's natural tendency to choose the novel object in a pair even after only brief familiarisation, a few seconds earlier, with the other object of the pair^{14,15}; consequently, the criterion of 90 correct choices in 100 trials is achieved extremely quickly, in this case in an average of 99 trials and 28 errors (see Table 1), or about one-third the time required to learn matching, that is, to choose the familiar object in the pair¹⁴.

The animals were then divided into four lesion groups of three monkeys each and given either an amygdalectomy, hippocampectomy, both combined, or neither. One-stage bilateral lesions were made aseptically while the animals were under Nembutal anaesthesia (35 mg per kg body weight). To remove the amygdaloid body, the frontotemporal junction was elevated slightly and the tissue medial to the anterior tip of the rhinal sulcus was entered with a small-gauge sucker; all gray matter rostral to the pes hippocampi (which afterwards lay exposed in the ventricle) and medial to the white matter of the temporal lobe was then ablated under visual control through an operating microscope. To remove the hippocampal formation, the occipitotemporal convexity was elevated slightly and the tissue medial to the rostral half of the occipitotemporal sulcus was entered with the sucker until the ventricle was opened; the entire hippocampal formation and much of the underlying fusiform-hippocampal gyrus was then ablated, again under visual control through the microscope, the upper ventricular surface serving as a readily identifiable dorsal boundary along the entire length of the removal. For the combined ablation, both of these surgical procedures were used in a single stage. Two weeks postoperatively the monkeys were retrained to criterion, with the outcome as shown in Table 1. Neither amygdalectomy alone nor hippocampectomy alone produced appreciable impairment, but the combination of the two removals yielded a severe effect: an abrupt drop to chance levels of performance followed by an average relearning score that was ten times the average for initial learning.

When the monkeys had regained criterion, their recognition ability was tested further with procedures adapted from a study by Gaffan¹². First, the delay between familiarisation and choice was lengthened in stages from the original 10 s delay to 30 s, then to 60 s, and finally to 120 s, each stage being tested for 100 trials. Second, the number of objects given for familiarisation before pairing each one with a novel object was increased in stages from the original single object to a series of three objects presented successively, then to a series of five objects, and finally to a series of ten objects; each of these stages was tested for 150 trials. The data in Table 1 indicate that, again, neither amygdalectomy nor hippocampectomy alone yielded more than a mild impairment. Each of these groups averaged 91% correct across the six testing conditions, as compared with an average of 97% correct for the normal controls. By contrast, the combination of the two lesions had a profound effect, yielding an average score of 60% correct, or just above chance. Since the animals had already regained the ability to perform the basic task, their sharp drop in performance with the longer delays and list lengths presumably represents a true memory loss rather than some other difficulty such as in visual perception or problem solving.

This striking deficit in monkeys after amygdalo-hippocampal removals is not limited to recognition memory, where the animal must remember whether or not a test object had been seen before. An equally severe impairment has been found in associative memory (B. Spiegler and M.M., unpublished), where the animal must remember on the basis of a single trial whether or not the test object had been rewarded before¹². On this more difficult, associative task, unlike the recognition task used here, amygdalectomy alone had a significantly greater effect than hippocampec-

Table 1 Effects of removal of amygdala and hippocampus on memory

Groups		Preoperative		Postoperative		Delays (% correct)			Objects (% correct)		
		Trials	Errors	Trials	Errors	30 s	60 s	120 s	3	5	10
Normal control	1	100	26	0	0	97	98	96	96	93	91
	2	80	28	0	0	99	100	98	97	97	94
	3	40	19	0	0	98	99	98	97	96	92
Amygdectomy	1	120	42	80	32	95	95	95	91	92	82
	2	100	27	340	85	91	89	92	88	87	77
	3	80	30	0	0	91	95	94	96	95	87
Hippocampectomy	1	60	17	80	22	98	93	94	95	92	84
	2	100	26	120	32	85	89	83	89	85	71
	3	120	31	20	4	98	99	95	95	92	88
Amygdectomy + hippocampectomy	1	210	49	760	179	79	65	65	62	64	59
	2	100	26	1,500	429*	64	59	63	60	55	61
	3	80	22	700	203	61	47	52	53	58	44
Group means											
Normal control		73	24	0	0	98	99	98	97	96	92
Amygdectomy		100	33	140	39	94	93	94	92	91	82
Hippocampectomy		93	25	73	19	94	94	91	93	90	81
Amygdectomy + hippocampectomy		130	32	987	270	68	57	60	58	59	55

Scores in preoperative and postoperative columns are the numbers of trials and errors preceding criterion of 90 correct choices of the novel object in 100 trials (delay following familiarisation with the other object in the pairs was 10 s). Scores in delays columns are percentage correct in 100 trials at each of three longer delays tested in succession at the rate of 20 trials per day, except for the longest delay (120 s) which was tested for 10 trials per day. Scores in objects columns are percentage correct in 150 trials for each of three multiple-object conditions tested in succession: in the first condition, three objects were presented for familiarisation, one at a time at 20 s intervals, and then re-presented in the same order, each being paired with a novel object, again at 20 s intervals; in the next condition, five objects were presented one at a time at 20 s intervals, and so on. Thirty trials were presented daily, ten sets of 3's or six sets of 5's or three sets of 10's. The minimum delay between familiarisation and choice was 60, 100, and 200 s for the three conditions, respectively. Scores for individual animals are shown in the upper part of the table, group means in the lower part. Histological examination indicated that the lesions were as intended except in animals 'hippocampectomy 2' and 'amygdectomy + hippocampectomy no. 2', both of which sustained, in addition to the planned removals, bilaterally asymmetrical damage to the ventral part of inferior temporal ('visual') cortex.

*Failed; final score, 85 correct in 100 trials.

tomy alone; but neither effect presaged the abrupt and permanent fall to chance levels of performance that followed the combined ablation.

The evidence that a severe memory disorder in monkeys can be produced only by the combined removal of amygdala and hippocampus seems at first surprising; it implies that these two structures, so unlike morphologically, may nonetheless serve as functional substitutes in a still undefined memory circuit. Yet this conclusion gains plausibility from recent anatomical studies in monkeys which show that the amygdala and hippocampus have many more inputs and outputs in common than was previously supposed. Both of these structures receive projections from many of the same frontal and temporal association areas, the amygdala receiving them directly^{16,17}, and the hippocampus indirectly through the entorhinal area¹⁸⁻²⁰; and both of these structures send projections, in turn, to many of the same diencephalic and basal forebrain areas, the amygdala via both the ventral amygdalofugal pathway and the stria terminalis²¹, and the hippocampus by way of the fornix²². Thus, while the amygdala and hippocampus undoubtedly serve different functions, they could still constitute alternative relays through which a particular group of cortical association areas could interact with a particular group of subcortical targets, such that only the combined removal of both relays would cause a complete disconnection.

Taken together, these behavioural and anatomical findings in monkeys open up a new possibility for helping to resolve the long-standing discrepancy between the animal and clinical literature on amnesia. To pursue this possibility, several kinds of additional investigations are needed. Among the most critical are: a determination of whether the severe memory impairment demonstrated here following combined amygdalo-hippocampal damage in monkeys transcends the visual modality, in keeping with the 'global amnesia' found in man; a reappraisal of the neuropathology in human amnesic cases with special attention to whether

there is indeed damage to the amygdaloid system as well as the hippocampal system; and quantification of the memory impairment in clinical cases, to determine whether differences in degree of amnesia might correlate with differences in amount of pathological involvement of these two systems.

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Thermoregulation and the maternal behaviour of the rat

THE time that a mother rat spends with her litter declines progressively during the first two postpartum weeks¹⁻³. In view of the increasing nutritional demands placed on her by the litter this declining nest-time seems paradoxical and suggests that factors other than those usually associated with the process of milk delivery may be in operation. The size and thermal output of the litter also increase, placing greater limits on maternal heat loss through the ventral skin while the mother is nesting. In this letter we report preliminary evidence supporting the hypothesis proposed by Croskerry² that thermoregulatory requirements increasingly limit the duration of nest bouts and total nest-time over the first 2 weeks.

During a study of nesting behaviour we noticed that nest time was negatively correlated with a substantial drop in daily maximum ambient temperature (Fig. 1). It is probable that room temperature was closely correlated with these changes in ambient as the room was not thermostatically controlled and the windows were usually open. For the 12 mothers, the correlation between mean nursing-time over days 2-4 of lactation and mean daily temperature (obtained from the local office of Environment Canada) was significant ($r = -0.90$, d.f. = 10, $P < 0.001$)^{3,4}.

In the same experiment we also noticed that at high ambient temperatures mothers would often groom the ventrum on leaving the nest. This may have been due simply to the incompatibility of nursing and grooming behaviours; the grooming which follows a nursing episode occurring because previously it had been inhibited by nursing. Alternatively the grooming may have been thermoregulatory and if so this suggests that the mother's temperature may have risen substantially during the nursing bout, as Hainsworth and Stricker⁵ have shown that female rats do not use this form of regulation until their central temperature reaches about 40 °C. On other occasions where ambient temperatures have been high (~80 °F) we noted that, on leaving the nest, mothers would adopt the supine posture which is a characteristic response to thermal stress. This behaviour is rarely seen in the non-nursing rat and it seems that the lactating rat may be attempting to achieve more effective cooling than would occur in the normal posture when the ventral surface is occluded by the floor of the cage. At ambient temperatures below 26 °C, 90% of heat loss occurs by convection, conduction and radiation from the body surface, evaporation accounting for the remaining 10% (ref. 6). Heat loss from the tail accounts for only about 5% of the total in these conditions⁷. Thus occlusion of the ventral surface by the floor of the cage or by the litter would be expected to have a substantial impact on thermoregulation.

These two observations strongly suggest that, at least when the environmental temperature is high, periods of nursing may terminate when the mother's ventral or perhaps her core temperature reaches a critical level, and we would expect that total nursing times would be shorter than they are when these thermoregulatory constraints do not exist.

Similar arguments may be invoked to account for the declining nest-time referred to above. When the litter provides a large sink for the absorption of heat (for example, when the young pups generate little heat of their own and their temperature falls rapidly toward ambient after the mother leaves the nest), the mother's critical temperature will be reached only relatively slowly. In such cases nest periods would be expected to be relatively long. On the other hand, as the litter grows and increases in mass, and as thermoregulatory mechanisms develop, the litter becomes a progressively smaller sink or even a source of heat. The

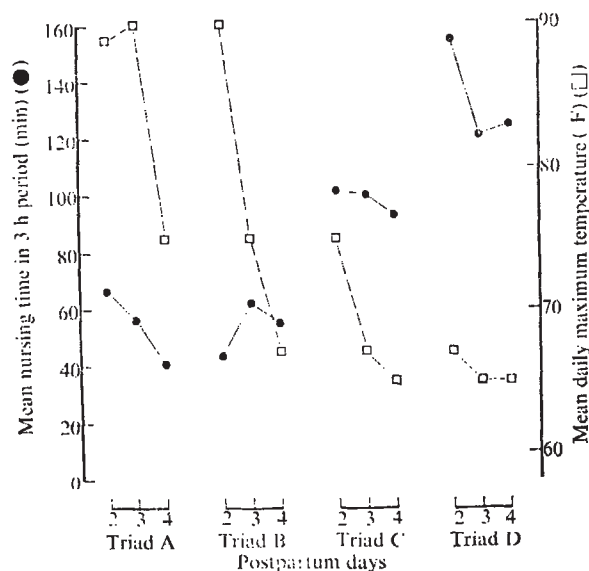
mother now will reach her critical temperature more quickly and nursing periods should be correspondingly shorter.

Such a mechanism of control of nesting behaviour would require first, that it is the mother who terminates the nursing bout and not the pups. From our own observations this always seems to be the case, and others have reported that pups put to an anaesthetised mother will continue to suckle for several hours⁸. Second, it must be possible for the mother rat to detect and respond to differences in peripheral skin temperature or core temperature. There is sufficient evidence that rats are able to discriminate relatively small excursions in peripheral temperature, and that behavioural adjustments are made to alleviate discomfort⁹⁻¹².

For such an account of maternal absence from the nest to be plausible it would be necessary to show that the core or skin temperature of the nursing mother rises substantially over a nursing episode and falls substantially when she leaves the nest. Accordingly in a pilot study thermistors were implanted in lactating mothers under ether anaesthesia on the second postpartum day; one beneath the ventral skin at the sternum and another in the peritoneal cavity near the left kidney. After a brief recovery period the mother was reunited with her litter and soon exhibited normal maternal behaviour. They were housed in a cage fitted with a continuous nest time recorder³. Beginning at noon on the seventh postpartum day recordings were made of the output of both thermistors and of the maternal nest-time. Figure 2 shows a 160-min period from one such record in which three nest episodes occur. Both ventral and peritoneal temperatures rise gradually over the nesting episodes and fall when the mother leaves the nest. The ventral temperature falls most abruptly; the total excursion is about 2.2 °C. The peritoneal temperature, although it descends more gradually, falls about 1.5 °C between nesting episodes. If this placement is representative of core temperature it suggests that substantial increases do occur during nursing.

These preliminary results support the proposition that nesting-time in the rat is limited by thermoregulatory considerations and provide a reasonable explanation for the paradoxical decline in nest-time observed with postpartum

Fig. 1 The relationship between ambient temperature (□) and nest-time (●). Each of the four groups consisted of a triad of mothers who delivered on the same day. Their pups were cross-fostered on day 1 so that each mother received three of her own pups and three pups from each of the other two mothers in the triad. Nesting time was observed for 3 d starting on day 2. The four triads delivered on successive days so that day 2 of triad B is day 3 of triad A, and so on.



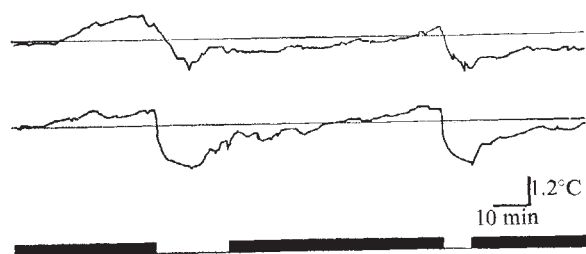


Fig. 2 *a*, Peritoneal temperature; *b*, ventral subcutaneous temperature. Heavy black bar indicates nesting episodes.

time. Extensive confirmation of these results will be provided in a forthcoming report of the role of hormones in nesting behaviour¹³.

These results can be used to explain some other features of maternal behaviour observed by us and others. For instance, the number of pups in the litter will determine the effective surface area to volume ratio of the litter and therefore the kinetics of heat exchange with the environment during maternal absence. The effective interface between mother and litter will also depend on the number in the litter and this may influence the rate or extent of temperature rise during nursing, although the effect would be offset to some extent by the lower plane of growth, and hence total mass, of large litters¹⁴. It would be predicted, then, that mothers spend more time with small litters than with large ones, and this, indeed, is the case^{1,15,16}. The same argument could also explain the observation that a mother will spend an amount of time with the litter that is appropriate to its age and not to her stage of lactation^{16,17}.

It would also be expected that maternal body weight would influence nest-time for two reasons. Heavier mothers would provide a more effective interface with the litter and, having themselves a lower surface area to volume ratio, would be less efficient at losing heat and should be more susceptible to hyperthermia. This would result in a higher rate of rise of maternal temperature which would reduce the duration of the nursing period. This seems to be the case. In a study of the influence of ad lib weight on maternal behaviour total nest-time was negatively correlated with maternal body weight at parturition ($r = -0.7$, d.f. = 9, $P < 0.05$) and in another in which maternal weight was increased by growth hormone treatment a correlation was also observed ($r = -0.62$, d.f. = 14, $P < 0.01$)⁴. Conversely, it might be expected that experimental reduction of maternal body weight would lead to longer nesting time. This expectation is borne out in a study by Massaro *et al.*¹⁸ and another by Lynch¹⁹. It should also be pointed out that the decline in nest-time during early lactation is accompanied by an increase in maternal body weight²⁰.

Clearly other factors must be active in terminating nursing episodes and it is not clear how thermoregulatory considerations might influence the return of the mother to her nest. (Pups emit ultrasonic cries which have been shown to elicit maternal search and retrieval behaviour^{21,22}). Nevertheless several features of nesting behaviour in the rat which are otherwise puzzling seem susceptible to explanations based on the mother's need to lose heat.

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Antigenic heterogeneity of metacyclic forms of *Trypanosoma brucei*

AFRICAN trypanosomiasis is a relapsing disease chiefly affecting man and his domestic stock. The infected mammal mounts a protective, trypanolytic immune response to the trypanosome's surface antigen, but a few parasites have already undergone antigenic variation by that time¹. Parasitaemic remissions and recrudescences occur as a seemingly inexhaustible series of surface variable antigen types (VATs) are expressed randomly by the flagellate^{2,3}. Reversion of the trypanosome population to a 'basic' antigen type at the end of its cyclical development in the tsetse fly's salivary glands has been suggested⁴⁻⁶. Prospects of vaccination, and of early serodiagnosis of trypanosomiasis, would be greatly improved by use of such a basic VAT. Specific immunofluorescence and immune lysis tests now make possible the identification of the VAT of individual trypanosomes¹. We have applied these techniques to metacyclic trypanosomes. Our results show that VAT heterogeneity is present in the metacyclic population.

We have transmitted through *Glossina morsitans morsitans* trypanosomes of the AnTat serodeme. This is a collection of VATs derived from a clone of *Trypanosoma brucei brucei* EATRO 1125 (ref. 7). Batches of 50-200 flies were fed, within 36 h of hatching from pupae, on CFLP mice infected with one of three VATs (AnTat 1, 8 or 9). The flies were maintained at $25 \pm 1^\circ\text{C}$ and $75 \pm 5\%$ relative humidity, and fed on rabbits during the 2 weeks following the infecting meal; subsequently on every second day the flies were encouraged to salivate (probe) on to heated microscope slides and to feed on clean test mice in order to determine which of the individual insects were infected. Metacyclic trypanosomes were then collected by allowing these individuals to probe into 1:1 Hanks' glucose saline: foetal calf serum warmed to $37-40^\circ\text{C}$. Metacyclic and bloodstream reference material was stored as acetone-fixed smears at -20°C (ref. 1) and as stabulates at -196°C (ref. 8). Cloning of trypanosomes, indirect immunofluorescence tests (IFT), immune trypanolysis (TL) and neutralisation of infectivity tests were carried out as described by Van Meirvenne *et al.*¹. After cyclical transmission, the antigenic composition of the clones was monitored by TL checks and neutralisation of developing heterotypes at every passage⁹.

In a preliminary cyclical transmission experiment⁹, marked VAT heterogeneity was found in the first patent parasitaemia in mice, suggesting that the metacyclic inoculum was already heterogeneous and/or that VAT diversity developed quickly after infection of the mammal. Further transmission experiments reported here support the

first interpretation. Data summarised in Table 1 show that the surface antigens carried by the metacyclics are also expressed by trypanosomes in a syringe-passaged mammal infection. Rabbit end-infection antisera collected 4–6 weeks after syringe infection with an AnTat clone contain lytic antibodies specific to more than 22 different variants of the serodeme. Such sera lysed most of the AnTat metacyclics tested—a result which demonstrates first, that the metacyclic phenotype is not confined to the tsetse fly and second, that the metacyclic VATs are possibly heterogeneous. Parallel controls were carried out by incubating metacyclics in fresh guinea pig serum and in anti-tsetse fly saliva rabbit serum. The absence of trypanolysis induced by the latter is the first evidence reported that vector antigens are not associated functionally with the metacyclic trypanosome's surface.

A pool of monospecific antisera to the VATs AnTats 1–22 (Table 1) induced the lysis of nearly 10% of the metacyclic trypanosomes collected on several occasions from different flies. These results give direct evidence for antigenic heterogeneity of the metacyclics and show that AnTats 1–22 are not major components of the metacyclic population in the salivary glands of the vector. In addition, day 7 sera collected from mice and rabbits bitten by infected flies lysed only 86% of metacyclics, suggesting that by day 7 the mammalian host had not yet produced antibodies against some of the metacyclic antigen types, probably because they represented minor variants in the metacyclic population.

Indirect IFT utilising the same day 7 sera were carried out on living metacyclics and confirmed the conclusions drawn from the immune lysis experiments. Thirty per cent of the metacyclics exhibited a bright fluorescence, whereas 58% were weakly labelled and 12% showed no fluorescence at all (Fig. 1). Such differences probably reflect the relative prevalence of the corresponding VATs in the metacyclic population—30% of the organisms belonging to predominant VATs, 58% carrying VATs against which the host had not yet mounted a marked immune response, 12% being minor variants. Controls were carried out with hyperimmune sera prepared against culture forms of *T. brucei*. Such sera contain antibodies to common antigens present on the surface at 'uncoated' stages of the life cycle, but no fluorescence was observed when these sera were applied to metacyclics in the indirect IFT. This result argues against the possibility that the weakly fluorescing trypanosomes were simply metacyclics which had only partially acquired (or had partially lost) the variable antigen-containing surface coat^{10,11} and that the negative organisms represented epimastigote or proventricular stages.



Fig. 1 Antigenic heterogeneity amongst *T. brucei* metacyclic trypanosomes as demonstrated by surface antigen-specific indirect IFT on living organisms. A marked positive reaction is shown by three trypanosomes, whereas the fourth is weakly labelled; others displayed no labelling (see text). Probed living metacyclics were incubated for 30 min at 0–4 °C in 0.1 ml 1:1 Hanks' solution:foetal calf serum with rabbit D7 MT (see Table 1) at dilution 1/20, washed twice for 5 min, incubated for 30 min at 0–4 °C in 0.1 ml buffer with FITC-conjugated goat anti-rabbit Ig (Pasteur Institute, Paris) diluted 1/200, and re-washed twice. Examination was with Ploem illumination (Leitz Ortholux II). For photography trypanosomes were fixed in 2% formalin.

To analyse the actual VAT composition of a metacyclic population, monospecific antisera against the different VATs would be necessary, and preparation of such antisera is now in progress using metacyclic-derived clones, of which 23 have so far been isolated in mice. One such clone (clone 19) has been adapted to mice by 3-d passaging, during which faster growing heterotypes were neutralised with antisera against AnTats 1–22. An antiserum raised against this clone lysed 22% of metacyclics and three out of nine of the other clones tested: these results indicate that clone 19 corresponds to a major metacyclic antigen type. Of the 23 clones isolated, only one has been identified as belonging to the previously demonstrated AnTat collection (that is, AnTats 1–22; refs 1 and 9). Thus, the metacyclic population would appear to contain at least two VATs.

The evidence presented above leads us to conclude that the metacyclics of *T. brucei* are heterogeneous with respect to the antigen type which they express and that reversion to a basic VAT at the metacyclic stage in the trypanosome's life cycle is not universal. The apparent homogeneity of metacyclic VAT reported for *T. brucei* by Jenni⁹ may have been due to his use of polyvalent 10-d antisera collected from rabbits bitten by infected flies on more than one occasion. Heterogeneity is consistent with the notorious absence of complete immune protection observed in the field, even in restricted areas where a limited collection of basic types could be expected to circulate.

This conclusion has important theoretical and practical overtones. If variable antigen heterogeneity occurs in the salivary glands of the vector, it is unlikely that antibody plays a necessary role in inducing antigenic variation in the mammal as has often been supposed. Indeed, it is difficult to imagine what stimulus could evoke the differentiation of several antigenic phenotypes within the confines of the salivary gland, a difficulty which might be resolved by conceding that antigenic variation may, after all, involve some genotypic change, or that a novel mechanism of biological variation should be considered. From the practical viewpoint, metacyclic heterogeneity makes the task of protecting against trypanosomiasis by vaccination all the more difficult.

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Table 1 Immune lysis of metacyclic trypanosomes (lysed/total)

Fly No.	Age	Control sera GPS	FRS	REIS	Antisera A 1–22	D7 MT
I2 B-8	32 d	0/14	0/10	7/10	3/10	14/16
I5 A-1	28 d	0/10		10/11	1/12	65/76
II5 A-10	29 d	0/6	0/3		3/6	7/8
III3 A2-5	38 d	2/100		70/70	14/169	
III3 A2-5	43 d	0/25		50/50	0/25	
Total		2/155	0/13	137/141	21/222	86/100
%		1.29		97.16	9.45	86.00

Living metacyclic trypanosomes were suspended in 20 µl guinea pig serum (GPS): 1 µl was mixed with 1 µl anti-serum, incubated as a hanging drop over a humid cavity slide at 26 °C for 2 h, and examined by phase microscopy (×40 objective). Antisera: fly rabbit serum (FRS), animal bitten every second day for 2 weeks by 172 non-infective tsetse, serum diluted one-third; pooled sera (REIS) from five rabbits syringe-infected with AnTat 1, 2, 3, 7 and 14, collected after 32–43 d, undiluted; pool of monospecific antisera (A 1–22) against AnTat 1–22, undiluted; sera from rabbits and mice 7 d after bite by infected flies (D7 MT). Results from individual sera applied to same metacyclic sample are summed (later bleedings from the same animals showed no increase in titre).

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An antiserum against 9,11-azo-15-hydroxyprosta-5,13-dienoic acid recognises and binds prostaglandin endoperoxides

THE prostaglandin endoperoxides PGG_2 and PGH_2 , derived from arachidonic acid, are transient regulators in mammalian cells¹⁻⁴. On formation, the endoperoxides are rapidly converted to $\text{PGF}_{2\alpha}$, PGE_2 , PGD_2 and their 15-keto-PG metabolites; to 12- α -hydroxy-5,8,10-heptadecatrienoic acid (HHT) and malonaldehyde⁵; to thromboxane A_2 (refs 6, 7); and to PGI_2 (prosta-cyclin or PGX ; ref. 8). The enzymatic composition of each tissue governs the nature and extent of these conversions. By their direct influence and as precursors for other prostaglandins, the endoperoxides are primary cellular regulators; unfortunately, their short half life and intrinsic instability makes direct determination of PGH_2 related phenomena difficult. By using as the hapten a stable mimic⁹, stereochemically similar to the physiologically authentic endoperoxides, we have prepared antibodies which recognise and bind PGG_2 and PGH_2 . Enzymatic and chemical conversion of the endoperoxides and their pharmacological influence on platelet aggregation *in vitro* are retarded by these antibodies.

9,11-Azo-15-hydroxyprosta-5,13-dienoic acid was conjugated to bovine serum albumin (BSA) with water soluble carbodiimide¹⁰. Sixteen to eighteen molecules of prostaglandin were attached to each molecule of BSA. After dialysis and lyophilisation, a portion (1 mg) of the conjugate was emulsified with 0.9% saline (1.0 ml) and Freund's complete adjuvant (1.0 ml), and the emulsion was injected intradermally at 30-40 sites on the flanks of albino New Zealand rabbits. At the first inoculation rabbits were injected intramuscularly with 1.0 ml of pertussis vaccine at four sites on their hindquarters. Booster injections of 0.2 mg of conjugate, emulsified in 0.9% saline (1.0 ml) and Freund's incomplete adjuvant (1.0 ml) were administered 4 weeks after the first inoculation and at 8-10 week intervals thereafter. Animals were bled 2-3 weeks after the boosting.

The cyclooxygenase enzyme of blood platelets transforms arachidonic acid into PGG_2 and PGH_2 (refs 1-4), which then promote platelet aggregation either directly^{11,12}, or after their conversion to thromboxane A_2 (refs 6, 7). PG endoperoxide binding antibodies were detected by measuring the influence of the mimicked PGH_2 antiserum on arachidonate-mediated platelet aggregation.

Platelet-rich plasma (PRP) 2 ml, containing either normal rabbit serum or PGH_2 mimic antiserum, was challenged with

arachidonic acid, PGH_2 , PGG_2 or 9,11-azo-15-hydroxyprosta-5,13-dienoic acid and the ensuing aggregations were monitored with a Payton aggregometer. Samples (0.1 ml) were drawn from the cuvette at 1-min intervals for radioimmunoassay of PGE_2 (ref. 10) and thromboxane B_2 (TXB_2)¹³.

Biochemical properties of 9,11-azo-15-hydroxyprosta-5,13-dienoic acid during *in vitro* platelet aggregations have been reported⁹. Antiserum prepared against a protein conjugate of 9,11-azo-15-hydroxyprosta-5,13-dienoic acid suppressed the hapten-induced aggregation of PRP in a concentration dependent fashion, verifying the presence of hapten binding antibodies.

Antiserum against 9,11-azo-15-hydroxyprosta-5,13-dienoic acid, the PGH_2 mimic, suppressed the initial rate, the time of onset and the maximal extent of arachidonate-mediated aggregation of PRP (Fig. 1). Both the rate and extent of TXB_2 and PGE_2 production declined in inverse proportion to the

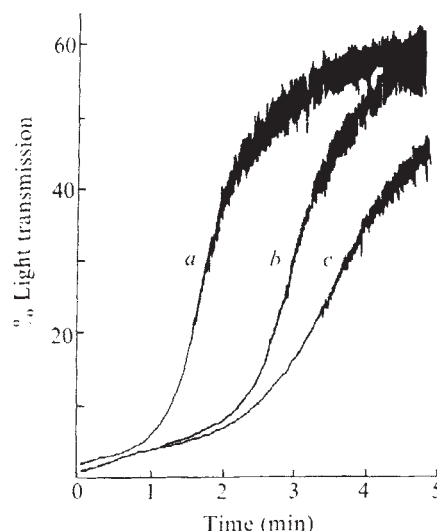


Fig. 1 An antiserum against the hapten, 9,11-azo-15-hydroxyprosta-5,13-dienoic acid, retards the aggregation of PRP by arachidonic acid. Platelet-rich plasma was prepared by centrifuging fresh, human whole blood collected over citrate (1 part 3.8% citrate, 10 parts blood) at 200g for 10 min at 25 °C. Two ml of platelet-rich plasma containing the amounts of normal rabbit serum or 9,11-azo-15-hydroxyprosta-5,13-dienoic acid antiserum indicated below were incubated at 37 °C for 2 min; then transferred to an aggregometer cuvette containing 800 µg of arachidonic acid. The PRP was stirred at 1,100 r.p.m. at 37 °C. A, 200 µl normal rabbit serum; b, 50 µl mimicked PGH_2 antiserum; c, 200 µl mimicked PGH_2 antiserum.

amount of antibody (Fig. 2). This decline is consistent with the hypothesis that the antibodies bound prostaglandin endoperoxides formed from arachidonic acid by platelet cyclooxygenase, and prevented their subsequent conversion into TXA_2 (TXB_2) and PGE_2 . Different Michaelis-Menten constants (K_m) for the PGE_2 isomerase and the thromboxane synthetase could explain an apparent difference in the relative extent of PGE_2 and TXB_2 suppression. A slow rate of chemical formation of PGE_2 from PGH_2 combined with a fast rate of enzymatic formation of TXA_2 from PGH_2 may also explain this apparent difference.

The mimicked antiserum inhibited arachidonic acid-induced aggregation competitively. Platelets continuously transform arachidonic acid into PG endoperoxides until the antibody combining sites become saturated. Once antibody saturation occurs PGG_2 and PGH_2 accumulate in the PRP and ultimately provoke aggregation. The endoperoxides may exert their effects directly as suggested by Needleman *et al.*^{11,12}; indirectly, after conversion to TXA_2 ; or compositely, through the influence of PGG_2 , PGH_2 and TXA_2 . The results do not permit discrimination among these alternatives.

The antibodies retarded only the second wave of adrenaline-induced aggregation revealing a specific involvement with the

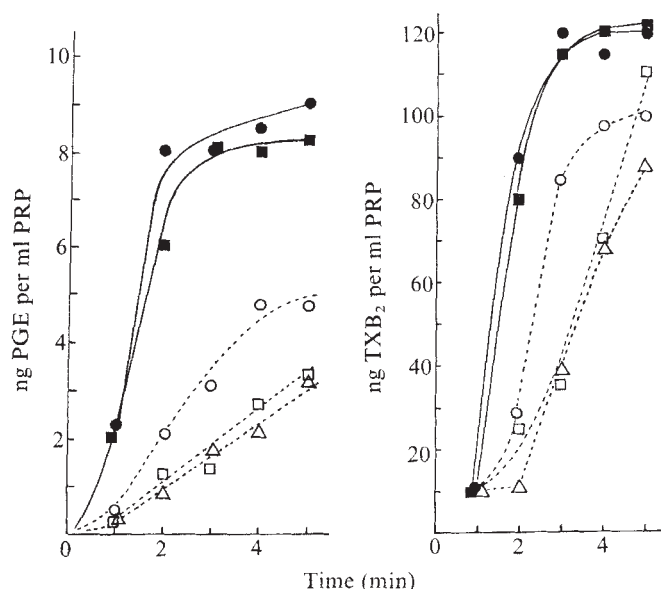
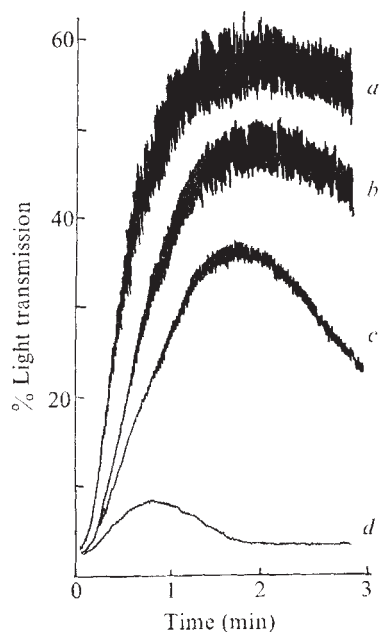


Fig. 2 An antiserum against the hapten, 9,11-azo-15-hydroxyprosta-5,13-dienoic acid, suppressed the formation of PGE_2 and TXB_2 during the aggregation of PRP with arachidonic acid. Two ml of platelet-rich plasma containing the amounts of normal rabbit serum or 9,11-azo-15-hydroxyprosta-5,13-dienoic acid antiserum indicated below were treated as in Fig. 1. Samples (0.1 ml) were withdrawn for the analysis of PGE_2 and TXB_2 by radioimmunoassay¹⁰. ●, 50 μl normal rabbit serum; ■, 100 μl normal rabbit serum; ○, 50 μl mimicked PGH_2 antiserum; □, 100 μl mimicked PGH_2 antiserum; △, 200 μl mimicked PGH_2 antiserum.

cyclooxygenase related aspect of platelet aggregation. The antibodies retarded aggregation induced directly with PGH_2 in a concentration-dependent fashion (Fig. 3). Analogous results were obtained for PGG_2 -induced aggregations. Similar stereochemical and conformational features between the 9–11 carbon region of PGH_2 and 9,11-azo endoperoxide mimic are critical to the successful generation of endoperoxide binding antibodies.

Fig. 3 An antiserum against the hapten 9,11-azo-15-hydroxyprosta-5,13-dienoic acid retards the aggregation of PRP by PGH_2 . One ml of platelet-rich plasma containing the amounts of normal rabbit serum or 9,11-azo-15-hydroxyprosta-5,13-dienoic acid antiserum incubated below was treated as in Fig. 1. PGH_2 (600 ng) was used to stimulate aggregation. *a*, 100 μl normal rabbit serum; *b*, 75 μl mimicked PGH_2 antiserum; *c*, 85 μl mimicked PGH_2 antiserum; *d*, 100 μl mimicked PGH_2 antiserum.



As PGG_2 and PGH_2 are identical in this respect it would be surprising if the mimicked antibodies recognised one endoperoxide but not the other. Quantitative differences between the association constants of the antibody for PGG_2 and PGH_2 because of structural differences at C-15 (PGG_2 , $-\text{OOH}$; PGH_2 , $-\text{OH}$) can not be determined experimentally by classical immunochemical methods because of the instability of the endoperoxides in an aqueous medium.

The same experiments repeated with antisera against PGE_2 , $\text{PGF}_{2\alpha}$ or TXB_2 gave results identical to the normal rabbit serum control, ruling out cross-reaction effects of these compounds, or nonspecific binding of PGH_2 to other prostaglandin antisera. Additionally, even at unusually high antiserum concentrations (1:4 to 1:10 dilutions) the PGH_2 antibodies had a low affinity for several prostaglandins and thromboxane B_2 . The antiserum at a 1:4 dilution bound $\text{PGF}_{2\alpha}$ (1.2%); PGE_2 (3.0%), TXB_2 (4.4%); 13,14-dihydro-15-keto- $\text{PGF}_{2\alpha}$ (0.8%); 6-keto- $\text{PGF}_{1\alpha}$ (0.5%) at concentrations of 1 ng ml^{-1} . The antibodies against PGH_2 also did not cross react significantly with TXA_2 . Concentrations of antiserum which inhibited PGH_2 -induced aggregations by 50% did not inhibit aggregations induced by TXA_2 generated *ex situ* from platelet microsomes and the same amount of PGH_1 .

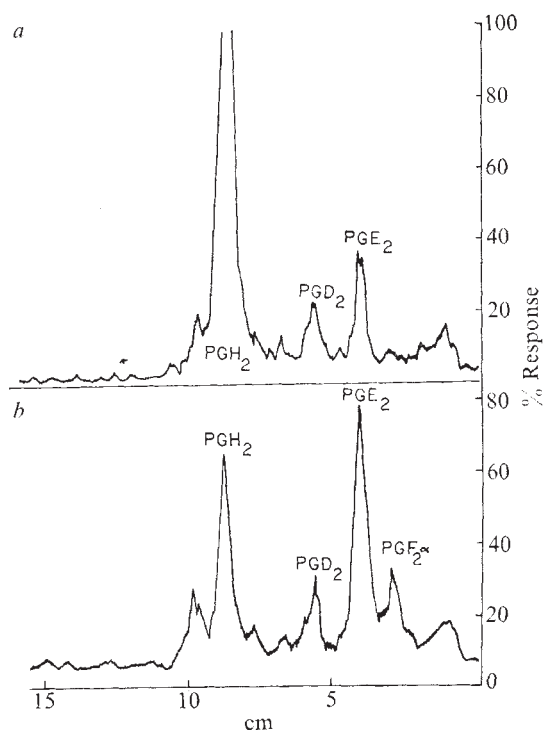


Fig. 4 An antiserum against the hapten, 9,11-azo-15-hydroxyprosta-5,13-dienoic acid retards the conversion of prostaglandin endoperoxide (PGH_2) into classical prostaglandins by serum proteins or hydrolysis. ^{14}C - PGH_2 was added to 500 μl of 0.1 M, phosphate buffer (pH 7.4, 0.9% NaCl) containing either 100 μl of normal rabbit serum or 100 μl of PGH_2 mimic antiserum. The samples were incubated at 4°C for 30 s and extracted with 6 volumes of ethyl acetate. After desiccation over Na_2SO_4 and evaporation to 50 μl under nitrogen, the ethyl acetate concentrates were applied to thin layer chromatography plates which were developed in the upper phase of 2,2,4-trimethylpentane:ethylacetate:acetic acid:water, 50:90:20:100. Radioactive zones were identified by comparison with known standards chromatographed on the same plate. *a*, Radiometric thin layer chromatographic [system AIX] profile resulting after the incubation of ^{14}C - PGH_2 with 5 parts phosphate buffer (0.1 M, pH 7.4, 0.9% NaCl) and 1 part mimicked PGH_2 antiserum. More than 95% of the PGH_2 was recovered intact after a 30-s incubation at 4°C . *b*, Radiometric thin layer chromatographic [system AIX] profile resulting after the incubation of ^{14}C - PGH_2 with 5 parts phosphate buffer (0.1 M, pH 7.4, 0.9% NaCl) and 1 part normal rabbit serum. Over 50% of the PGH_2 was converted to $\text{PGF}_{2\alpha}$, PGE_2 and PGD_2 after a 30-s incubation at 4°C .

Binding of PGH_2 was verified directly. ^{14}C - PGH_2 (6×10^4 d.p.m.) prepared biosynthetically from ^{14}C -arachidonic acid and sheep seminal vesicle acetone pentane powder¹⁴ was incubated at 4°C , with phosphate buffer (pH 7.4) solutions of normal rabbit serum or PGH_2 antiserum and at intervals extracted with 6 volumes of ethyl acetate. The ethyl acetate extracts were subjected to radiometric thin layer chromatography. Radioactive zones were identified by comparison with known standards chromatographed on the same plate.

Over 50% of the ^{14}C - PGH_2 was converted to PGE_2 , $\text{PGF}_{2\alpha}$ or PGD_2 during a 30-s incubation with normal rabbit serum; however the serum of rabbits inoculated against the PGH_2 mimic showed less than 5% conversion (Fig. 4). Incubations for 10 min showed 100% conversion of PGH_2 by the control and only 25% conversion by the PGH_2 mimic antiserum. The antibodies cannot stabilise PGH_2 indefinitely in an aqueous environment since the antibody-hapten interaction is an equilibrium phenomenon. When the PGH_2 dissociates from the antibody it has a finite chance to decompose before it encounters another antibody molecule. The average intrinsic association constant (K_0) and the concentration of antibody combining sites (A_0) govern the stability of PGH_2 in aqueous solutions of antibody.

The short half life and chemical instability of the prostaglandin endoperoxide, PGH_2 , have previously excluded the preparation of antibodies against this hapten. A novel approach was taken to circumvent this problem. Essentially the approach lies in regarding cross reaction as a favourable property, rather than an unfavourable one. Sela¹⁵ has summarised the characteristics which confer immunopotency and immunodominance to antigens. By exploiting a stable hapten mimic, immunologically similar to the authentic isosteric counterpart we have elicited antibodies with the ability to bind PGH_2 . The antibodies retard arachidonate- or PG endoperoxide-induced platelet aggregation and the consequent production of thromboxane B_2 and PGE_2 , as well as chemical and enzymatic conversion of PGH_2 into classical prostaglandins. This antiserum should be a valuable, specific probe for dissecting extracellular phenomena regulated by PGH_2 . The hapten mimic selected must coincide closely in molecular geometry and electronic density to the authentic hapten to be successful.

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Receptor interactions on the membrane of resting and activated B cells

LIPOPOLYSACCHARIDE (LPS) activates one-third of the B cells in mouse spleen, inducing proliferation and maturation to immunoglobulin secretion¹. The surface structure functioning as receptor in the triggering process is specific for the lipid A moiety of LPS², and is encoded by a gene mapped on chromosome 4 (ref. 3), for which two nonfunctional alleles are known (refs 4, 5 and A. C. and T. Meo, in preparation). We have recently described an antiserum which seemed specific for that LPS receptor (LPSR) on the basis of morphological, functional and genetic evidence^{6,7}. The polyclonality of B cell responses to LPS⁸ has excluded the participation of immunoglobulin combining sites in the process of triggering. The existence of two LPS non-responder mutants, which display normal triggering behaviour with other reagents and a normal complement of immunoglobulin positive cells⁹, also suggested that LPS activation proceeds independently of surface immunoglobulin receptors. The finding of the inhibition of LPS activation by anti-immunoglobulin antibodies, however, suggested that mitogen receptors and surface immunoglobulins could be interacting on the B-cell membrane¹⁰. We report here direct morphological evidence that supports the existence of such interactions.

Membrane molecules when reacted with a multivalent ligand such as lectins or antibodies, are redistributed into polar caps through an energy-dependent process (see ref. 11 for review). This redistribution involves the molecules directly reacting with the ligand, as well as other molecules which are physically connected with them, or which interact as a consequence of the reaction of the former molecules with the ligand. In the first case, one observes bidirectional concomitant redistribution of the two molecules ('co-capping'), whereas in the second case the co-capping is more likely to be unidirectional. We made use of this phenomenon to ascertain relationships and/or interactions at the membrane level between four different surface molecules of murine B cells, LPSR, IgM and IgD surface immunoglobulins and Fc receptors (FcR).

The technique used was immunofluorescence. The specific anti- μ chain antiserum was directly labelled with fluorochromes using the method of Cebra and Goldstein¹². The anti- δ chain allotype antiserum of the same kind as those described by Goding *et al.*¹³, absorbed on normal mouse IgM and on spleen cells from LPS high responder mice, was used in indirect immunofluorescence labelled with trinitrophenol (TNP)¹⁴ followed by fluorochrome conjugated anti-DNP antibodies. The anti-LPSR antisera were prepared by immunising rabbits with LPS-high responder (C3H/Tif) splenic B cells and made specific by absorption in LPS non-responder (C3H/HeJ) mice. These antisera detect a surface marker(s) on LPS-reactive cells from all LPS-responder strains, and fail to stain any cell in the two available LPS-nonresponder strains. Genetic analysis indicates that this marker(s) is inherited with LPS-responsiveness. Furthermore, the binding of the antiserum to high responder B cells is specifically inhibited by a triggering concentration of lipid A, and an IgG fraction of the antiserum shows a dose dependent mitogenic activity on splenic B cells from LPS responder strains. On the basis of these and other results^{6,7}, we have concluded that such antisera recognise the triggering receptor for LPS. An IgG fraction prepared from the antiserum to high responder cells, absorbed with normal mouse IgM, was used in indirect immunofluorescence either labelled with TNP¹⁴ followed by fluorescent anti-DNP antibodies, or unlabelled and detected by fluorescent anti-rabbit IgG antibodies. Fc receptors were detected with fluorescent rabbit IgG anti-rabbit L chain allotype com-

plexes¹⁵ of a specificity so as not to interfere with the allotypic specificity of the rabbit antisera used throughout the experiments.

Double staining of two different markers was performed in all possible combinations. The first reagent was always used in conditions allowing redistribution of reacted molecules, that is, at 37 °C and in the absence of inhibitors; the second reagent was used in the cold in the presence of concentrations of sodium azide which completely inhibit energy-dependent membrane movement^{16,17}. The results of these experiments are shown in Tables 1 and 2.

In resting B cells, the following observations are noteworthy. Capping of surface IgD molecules results in co-capping of LPSR in every cell expressing these two structures, without significant redistribution of surface IgM on FcR. Capping of LPSR results in concomitant redistribution of surface IgM in roughly two-thirds of the cells, and of FcR on about one-third. As described previously¹⁵, capping of IgM results in complete co-capping of FcR on the vast majority of the cells, while capping of IgD does not. All these concomitant redistributions are in every case unidirectional. It seems therefore that all these surface markers are in fact distinct molecules, not physically linked to each other in the plane of the membrane. On the other hand, these observations point to the existence of a chain of surface structures on the B-cell membrane, arranged in the following hierarchical manner: IgD, LPSR, IgM, FcR.

To ascertain whether activation of the cells would result in changes in the expression and/or interactions of these molecules, we exposed mouse spleen cells for 12 h to triggering concentration of LPS and, as a control, of the mitogen from *Nocardia*¹⁸, and repeated these experiments. No

Table 2 Concomitant redistribution of IgM, IgD, LPSR and FcR on the membrane of resting and activated mouse spleen lymphocytes

Staining combination*	% cells with coincident cap†			
	No mitogen‡	LPS 50 µg ml ⁻¹ ‡	No mitogen‡	<i>Nocardia</i> 4 µg ml ⁻¹ ‡
µ→δ	4.8	1.5	ND	ND
δ→µ	7.0	36.7	6.7	28.9
µ→LPS-R	<0.5	<0.5	ND	ND
LPS-R→µ	68.9	34.8	65.7	63.6
δ→LPS-R	98.5	97.5	ND	ND
LPS-R→δ	2.8	3.0	ND	ND
µ→FcR	91.0	71.4	90.0	65.0
FcR→µ	<0.5	<0.5	ND	ND
δ→FcR	6.7	21.6	4.5	14.9
FcR→δ	0.5	<0.5	ND	ND
LPS-R→FcR	27.6	11.6	31.4	25.0
FcR→LPS-R	<0.5	<0.5	ND	ND

*Staining combinations as indicated in footnote to Table 1. First reagent used in 'capping' conditions; second reagent, labelled with a different fluorochrome, used in the cold and in the presence of 20 mM sodium azide.

†Cells on which the molecules detected by the second reagent are redistributed as a consequence of the capping of the molecules detected by the first reagent. Values are expressed as % co-caps out of all cells bearing the two surface markers.

‡Spleen cells from C3H.SW mice are cultured for 12 h in RPMI-1640 supplemented with HEPES, glutamine, antibiotics, 2-mercaptoethanol and 5% FCS.

ND, not determined.

drastic changes could be detected in the expression of these surface markers in the activated cells as far as the frequency of positive cells and the intensity of the fluorescent staining are concerned (Table 1). As we have shown previously⁷, there is no significant inhibition of the binding of anti-LPSR antibodies to cells exposed to LPS, once they have been washed free of LPS.

On the other hand, the molecular reactions revealed by redistribution are extensively altered (Table 2). Thus, in about one-third of the cells redistribution of IgD unidirectionally affects both IgM molecules, and to a lesser extent, FcR. Conversely, in a significant proportion of cells the interaction of LPSR with IgM, and of both LPSR and IgM with FcR is lost. Again, the hierarchy referred to above is kept, and no co-capping is observed in the reverse direction. The IgD-IgM co-capping in activated B cells seems to be a general phenomenon, since we also observed it, with no changes in the pattern of LPSR redistribution, in B cells activated by *Nocardia* mitogen (NOC), which certainly uses a triggering receptor distinct from LPSR (Table 2).

Since the present techniques limit to two the types of structures one can observe simultaneously, we do not know whether all those triggering-derived events take place in the same cell. Furthermore, as cells are asynchronously activated in these conditions (W. Lernhardt, personal communication), it is likely that we are observing cells at different stages along the activation pathway. It is conceivable that distinct relationships amongst membrane molecules are determined by the position of the cell in the mytotic cycle, and this possibility would account for the observed heterogeneity of the cells according to the membrane interactions we have here defined.

It is apparently established, however, that mitogen receptors and immunoglobulin receptors on the resting B cell membrane are functionally related, in ways which permit precise interactions when either of the surface molecules react with their ligand. This observation complements previous evidence demonstrating that both types of receptors are necessarily required for specific induction of B cells¹⁹, and substantiates postulates of a 'receptor complex' involving mitogen receptors and surface immunoglobulins¹⁹. Furthermore, the selectivity of the type of interactions with

Table 1 Expression of IgM, IgD, LPSR and FcR on resting and activated mouse spleen lymphocytes

Surface markers	12 h at 37 °C -LPS	12 h at 37 °C +LPS
µ ⁺	50.9% of total cells	46.7% total cells
δ ⁺	28.5% "	30.8% "
LPS-R ⁺	20.9% "	19.2% "
µ ⁺ δ ⁺	59% total IgM cells	58.8% total IgM cells
µ ⁺ LPS-R ⁺	40.9% of IgM cells	41.2% of IgM cells
δ ⁺ LPS-R ⁺	88% of IgD cells	85% of IgD cells
µ ⁺ FcR ⁺	93% of IgM cells	94.6% of IgM cells
δ ⁺ FcR ⁺	93.5% of IgD cells	92.1% of IgD cells
LPS-R ⁺ -FcR ⁺	91% of LPS-R ⁺ cells	87% of LPS-R ⁺ cells
LPS-R non-µ	8.6% "	9.2% "
LPS-R non-δ	6.8% "	5.9% "

Living cells in suspensions were exposed to suitable concentrations of the various reagents for 30 min in an ice bath, washed three times with buffered saline solution containing 10% foetal calf serum and 20 mM sodium azide, and treated with a second reagent labelled with a different fluorochrome. After washings, the samples were viewed under a fluorescence microscope (Zeiss Photomikroskop II or Leitz Orthoplan) equipped with vertical illuminator and combinations of filters and dichroic mirrors specific for fluorescein or rhodamine. Samples were observed in phase contrast to record the total number of cells in a microscopic field, and in fluorescence to detect labelled cells. The staining combinations were as follows: Tetramethylrhodamine (TRITC) rabbit anti-µ+(TNP-anti-δ+Fluorescein (FITC) goat anti-DNP antibodies). TRITC rabbit anti-µ+(TNP-rabbit anti-LPSR+FITC goat anti-DNP) (TNP-mouse anti-δ+FITC goat anti-DNP)+(rabbit anti-LPSR+TRITC sheep anti-rabbit IgG). FITC rabbit (allotype b 4, 4) anti-µ+(complexes Rabbit IgG allotype b 5, 5+TRITC rabbit anti-b 5). (TNP-mouse anti-δ+FITC goat anti-TNP)+(complexes as above (TNP-rabbit (allotype b 4, 4) anti-LPSR+FITC goat anti-DNP) + complexes as above. The anti-δ allotype antiserum was prepared in CWB/13 mice (H-2b, Ig-1b) by multiple intraperitoneal injections of C3H/HeJ (H-2k, Ig-1a) spleen cells. Because of the presence of anti-H-2k antibodies, the antiserum was used in these experiments on spleen cells from C3H.SW mice (H-2b, Ig-1a). The IgG fraction of the antiserum was absorbed on CWB/13 spleen cells, and on a 19S fraction of pooled sera from mice of Ig 1a allotype.

regard to the class of immunoglobulin receptor may lead to an understanding of the functional significance of the simultaneous expression of two immunoglobulin classes by the same cell²⁰.

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Evidence of fusion between host and donor myoblasts in skeletal muscle grafts

THE skeletal muscle fibre is a long, multinucleated cell formed by fusion of mononuclear precursor cells (myoblasts) with one another¹⁻³ and with muscle cells in various stages of maturity^{4,5}. In subjects suffering from inherited recessive myopathies, muscle function might be restored if normal myoblasts could be made to fuse with defective muscle fibres during muscle growth or regeneration. Such a method of introducing new genetic information into muscle fibres requires that myoblasts can enter a region of growing or regenerating muscle, and that these myoblasts can participate in the formation of new muscle fibres by fusion with locally derived cells. Recently we have shown, using isoenzymes of malate dehydrogenase as markers, that host muscle cell precursors can enter muscle grafts⁶. Here we present evidence, based on the use of a more sensitive isoenzyme marker, that fusion can occur between host and donor muscle cell precursors.

Grafts have been made between two inbred strains of mice (AKR and C3H), which are each homozygous for different allelic forms of glucose-6-phosphate isomerase (GPI: EC 5.3.1.9). GPI is readily detectable in many tissues including muscle. The active enzyme is a dimer formed in the cytoplasm by association of two subunits which are both coded for at the same gene locus. Where grafts are made between two mouse strains each homozygous for a different isoenzyme form of GPI, the presence of a particular isoenzyme reflects the presence of a nucleus of the correspond-

ing mouse strain. 'Hybrid' isoenzyme (consisting of one subunit of each of the two allelic types) can be formed in such grafts only when nuclei of both homozygous strains are present within the cytoplasm of the same cell, that is, in multinucleate cells such as muscle fibres. The formation of hybrid isoenzyme by cooperation between two different types of homozygous nuclei *in vivo* has only been demonstrated in muscles from mice which have developed from mixtures of morula cells of two different strains^{2,7}.

In the present study we have grafted minced skeletal muscle¹⁵ from one mouse strain into immunologically tolerant hosts of another strain. Tissues at the graft site have been shown to contain both host and donor isoenzyme, and in two instances, hybrid isoenzyme. The presence of this hybrid indicates that host and donor myoblasts can fuse to form mosaic muscle fibres, and thus that myoblasts from the host can enter the grafted (donor) tissue.

Grafts of minced tibialis anterior muscle were made into the anterior tibial compartment of the legs of 6-8-week-old host animals. To render host animals tolerant to the donor strain, they were injected intraperitoneally at birth with 10⁸ lymphoid cells obtained from spleens and lymph nodes of hybrid C3H×AKR mice⁸. Seventy-two grafts in all were made from AKR donors into tolerant C3H hosts, and 42 from C3H donors into tolerant AKR hosts: 18 AKR and 15 C3H grafts were made into C3H and AKR animals, respectively, in which no attempt had been made to induce tolerance. Grafts of mixed (AKR+C3H) muscle mince were made into 28 C3H and 12 AKR animals in which tolerance had been induced, and into three athymic *nu/nu* mice. At times between days 1 and 179, mice were killed and perfused with 0.9 w/v NaCl to flush host blood cells from the grafts. The tissue at the site of the muscle graft was removed and frozen in isopentane in liquid nitrogen. Frozen sections (5 µm) of each graft were cut, every tenth section being taken on to a glass slide and stained with haematoxylin and eosin. The relative proportions of muscle and other tissues were assessed visually in step sections, and in specifically mentioned grafts the amounts were also estimated by random spot counting⁹. Some 50-70 intervening sections were collected on a piece of filter paper (1×2 mm) which was inserted into an 11% starch gel (35×100 mm) (made in 0.01 M phosphate buffer, pH 6.5), and electrophoresed with 0.2 M phosphate bridge buffer, pH 6.5, for 7 h at 20 V cm⁻¹. The starch gels were then cut horizontally and overlaid with 7 ml 1% agar gel containing 1.5 ml 0.36 M Tris-HCl, pH 8.0, 0.5 ml 0.25 M MgCl₂, 1.5 mg fructose-6-phosphate, 2.5 mg NADP, 15 units of glucose-6-phosphate dehydrogenase, 1 mg nitro blue tetrazolium and 0.125 mg phenazine methosulphate. Gels were incubated at 37 °C for 15-20 min. The bands of formazan dye deposition, which indicated the position of the GPI isoenzyme, were photographed and, where necessary, optical density scans were made on the photographs. As standards, frozen sections were cut from separate blocks of C3H and AKR muscle (stored for up to 2 months at -70 °C) and put together on a single piece of filter paper (1×2 mm). Isoenzymes from such mixtures, even after repeated freezing and thawing, separated into two distinct bands on electrophoresis.

In minced muscle grafts sarcoplasmic structure was rapidly lost, and most muscle nuclei seemed to undergo pyknosis, fragmentation and lysis. At the edge of the graft, histiocytes, neutrophil polymorphonuclear leucocytes, lymphocytes and other unidentified mononuclear cells were initially conspicuous. By day 5 myotubes were found, and by day 20 (in tolerant hosts) there were many new mature muscle fibres; these tended to be irregularly orientated and to have abundant sarcoplasm and central nuclei. Myotubes were presumably derived from muscle cell precursors among the mononuclear cells seen initially at the edge of the graft; for it is widely held (for review see ref. 10) that myotubes

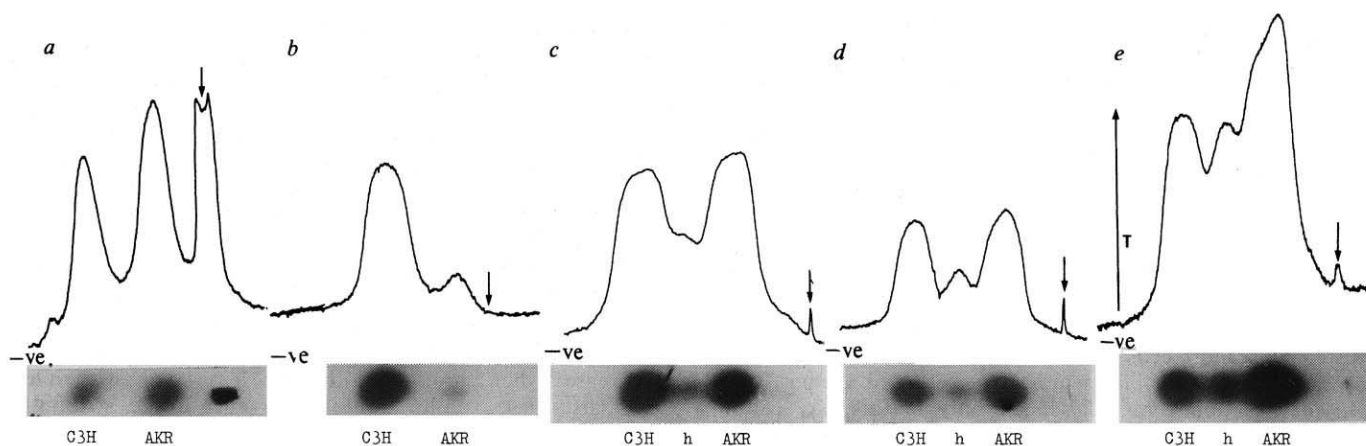


Fig. 1 Starch gel electrophoresis patterns of GPI isoenzymes derived from frozen sections of minced tibialis anterior muscle grafts. Optical density scans of photonegatives of the starch gels show the bands as peaks of optical transmission (T). The isoenzymes all migrate towards the negative electrode from the point of application (marked $\downarrow$) of the frozen sections. *a*, Mixture of frozen sections from AKR and C3H adult tibialis anterior muscles, run as a standard. Note clear separation into discrete bands of AKR and C3H isoenzymes. (The large peak at the point of application was due to damage to the gel.) *b*, Neonatal C3H muscle grafted into tolerant AKR host (day 41). On microscopic examination this graft was found to contain abundant (70%) new muscle. Note donor (C3H) isoenzyme in excess of host (AKR). *c*, Adult C3H muscle grafted into tolerant AKR host (day 100). The graft contained 25% new muscle. Note small band of hybrid isoenzyme (h). On the scan this is apparent as a rounded shoulder on the leading (C3H isoenzyme) peak. This hybrid isoenzyme indicates that individual muscle fibres containing both AKR (host) and C3H (donor) muscle nuclei were present in the graft. *d*, Mixed neonatal AKR + C3H muscle grafted into tolerant AKR host (day 57). The graft contained 30% new muscle. Note hybrid isoenzyme (h), indicative of the presence of individual muscle fibres containing both AKR and C3H nuclei. *e*, Mixed adult AKR + C3H muscle grafted into athymic *nu/nu* mouse (day 50). The graft contained 30% new muscle. Note hybrid isoenzyme (h): this indicates the presence of individual muscle fibres containing C3H nuclei together with AKR and/or *nu/nu* nuclei (of AKR isoenzyme type).

are formed by fusion of precursors derived either from 'satellite' cells or from segregated nuclei of muscle fibres. Grafts became stable in their composition by day 20. Thirty-three grafts, made into host animals where tolerance was not induced, and examined 20 d or more after implantation, contained host isoenzyme with a trace of donor in a single instance. Thirty of the 33 grafts were composed of fibrofatty tissue; three contained a few muscle fibres. These morphological findings accord with other studies¹¹⁻¹³, but contrast with one report¹⁴ of nine successfully regenerated muscles in 50 grafts made between Swiss White and 129/Rej mice. In these nine grafts, the microscopic examination of which was not reported, only donor isoenzyme was found. The absence of host isoenzyme is difficult to explain, but the success of these grafts could reflect an unrecognised histocompatibility between the strains of mice used.

In grafts made into tolerant hosts (over 20 d old) (see Fig. 1 and Table 1) we found both host and donor isoenzyme. In 13 of these grafts which lacked muscle, but which were composed of fibrofatty connective tissue, host and donor, but no hybrid, isoenzymes were found. The remaining grafts contained new muscle, which could have

been donor, or host, or donor and host. The donor contributions was probably the greater, as donor isoenzymes usually predominated in grafts which contained much new muscle.

Hybrid isoenzyme was found in two out of 22 grafts made into tolerant AKR hosts, but in none of the 49 AKR grafts made into tolerant C3H hosts (see Table 1 and Fig. 1). The two grafts in which isoenzyme was found contained 25% and 75% new muscle. The presence of hybrid isoenzyme indicates the presence in these grafts either of multinucleate cells (for example, muscle fibres) containing both host and donor nuclei, or of cells containing hybrid (AKR $\times$ C3H) nuclei. The simplest explanation for the presence of hybrid isoenzyme is the entry of host muscle-cell precursors and their fusion with donor muscle-cell precursors within the graft. It could be argued, however, that the hybrid isoenzyme might have been derived from hybrid (AKR $\times$ C3H) cells descended from lymphocytes originally injected into AKR hosts at birth to induce tolerance. This explanation is unlikely. Thus, although hybrid isoenzyme was certainly present in the spleens of tolerant host animals, only trace amounts were found in mesenteric lymph nodes, and the isoenzyme was absent from thymus, bone marrow, blood or liver. The foci of lymphoid cells found by microscopic examination in both grafts seem too small to account for the hybrid isoenzyme found; for in five other grafts in which lymphoid cells were abundant, hybrid isoenzyme was not detected.

Hybrid isoenzyme in grafts was thus almost certainly of muscle origin. To investigate this, we implanted mixtures of AKR and C3H minced muscles into 3 *nu/nu* athymic mice. These mice were not inbred, two being homozygous for AKR and one for C3H type isoenzyme. At day 50 all three grafts (containing 30, 30 and 50% new muscle) yielded hybrid isoenzyme; this could not, incidentally, have been derived from injected hybrid lymphoid cells, for athymic *nu/nu* mice readily accepted grafts without the induction of tolerance.

These findings indicate that fusion of two different strains of myoblast can occur readily in certain conditions. Where we failed to obtain hybrid isoenzyme in grafts, we must assume either that some factor prevented fusion or that one or other strains of myoblast was lacking from the graft. In

Table 1 Occurrence of hybrid isoenzyme in various minced tibialis anterior muscle grafts (over 19 d old) made into tolerant host mice

Donor strain (6 weeks old) or neonatal	Host strain	No. of grafts examined	Grafts containing more than 10% regenerated muscle No. of grafts	No. of grafts containing hybrid isoenzyme
Neonatal AKR	C3H	4	1	0
Neonatal C3H	AKR	7	7	0
AKR	C3H	49	10	0
C3H	AKR	22	19	2
Neonatal (AKR + C3H)	C3H	4	4	0
Neonatal (AKR + C3H)	AKR	8	8	2
(AKR + C3H)	C3H	24	7	0
(AKR + C3H)	AKR	4	4	1
(AKR + C3H)	nude	3	3	3

favour of the first possibility, we found hybrid isoenzymes in none of 28 mixed (C3H+AKR) muscle grafts made into tolerant C3H hosts; but we did find them in three (all containing 30% new muscle) out of 12 similar grafts made into tolerant AKR hosts, as well as in three out of three grafts made in *nu/nu* hosts; that is, the strain of the host animal does seem to influence the frequency with which fusion occurs in mixed muscle grafts. No hybrid isoenzyme, incidentally, was detected in any grafts aged less than 50 d.

Our experiments indicate that host cells always enter minced muscle grafts made between different strains of mice. With the marker isoenzyme used we can not tell how often these host cells included myoblasts. However, on two occasions host myoblasts must have entered the graft and fused with donor myoblasts, for only in this way can we explain the presence of hybrid isoenzyme. With a view, therefore, to introducing normal muscle cells into subjects with myopathies, we must next discover how to promote the fusion of two strains of myoblast in areas of muscle cell growth.

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Brain development influences the appearance of glial factor-like activity in rat brain primary cultures

THE development of the nervous system in mammals is thought to be a complex process which involves the orderly expression of a pattern of signals and events. The understanding of how development is controlled requires the identification of the biochemical events which are causally related to this process. Established cultured cell lines provide useful model systems which have yet to be validated by careful examination in conditions more closely related to those *in vivo*. We have shown previously that established glial cell lines release a macromolecular factor(s) which can induce morphological differentiation of neuroblastoma cells¹ and this glial factor(s) is distinct from the well characterised nerve growth factor². Here we report that glial factor (GF) activity is not confined to established cell lines, as a similar activity is also detected in the medium conditioned by certain primary cultures of rat brain. We further demonstrate that there is a correlation between the presence of GF activity in the medium from cultures of various brain regions and the age of the animal at the autopsy from which the primary culture was derived. Our results provide experimental evidence that primary cultures initiated at different developmental stages can parallel the cellular and biochemical development of the brain *in vivo*.

Using the test system shown in Fig. 1, GF-like activity has been investigated in the conditioned medium from primary cultures of brain and other tissues (Table 1). The level of GF-like activity in primary cultures initiated from adult rat brain is found to be two- to threefold higher than that detected in conditioned medium from C6 glioma cells or from primary cultures derived from 5-d-old rats. The media conditioned by primary cultures of kidney, lung, heart or liver derived from 5-d-old rats have much lower activity. (Primary cultures of the above tissues from adult animals are not yet possible.) Since the protein-DNA ratio is twofold higher in liver cultures than in the corresponding brain cultures, and this ratio has a slight tendency to decrease when brain cultures are initiated with older animals, these results would seem even more striking if based on the amount of protein per culture rather than DNA.

Following these preliminary results a more detailed study was undertaken to trace the course of detectable GF-like activity during pre- and postnatal brain development. As shown in Fig. 2, very little activity is found in the medium conditioned by primary cultures of embryos or young rats up to 3 d of age. A very marked (3-4-fold) increase in GF-like activity is found, however, between postnatal days 3 and 5. As also shown in Fig. 2, the increase in activity found in cultures derived from whole brain is paralleled by increases in cultures initiated from cerebral cortex or cerebellum. No such sudden change in activity is detectable in the medium

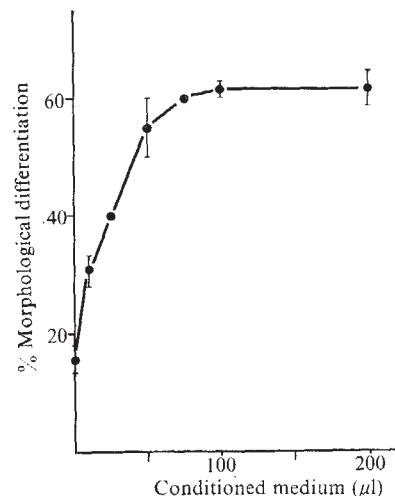


Fig. 1 Dose-response relationship between the amount of conditioned medium tested and the extent of morphological differentiation of neuroblastoma cells. The medium had been conditioned by a primary culture of the cerebral cortex from 11-d-old rats. In each figure vertical bars indicate the s.e.m. when it exceeds the size of the symbol. GF activity was determined in a neurite outgrowth bioassay using mouse neuroblastoma cells, clone NB₂A. The assay was a more sensitive modification of that reported earlier¹. Culture dishes (35 mm) were inoculated with 40,000 EDTA-dislodged neuroblastoma cells and incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS at 37 °C in a humidified 10% CO₂-90% air atmosphere. The medium was replaced 16-18 h later by DMEM containing 0.5-0.75% FCS which had not been heat inactivated, but dialysed exhaustively against 0.9% NaCl solution, filter-sterilised and frozen. After 22 h the bioassay was carried out by adding the test sample (10-100 μl) in a final volume of 2.0 ml DMEM. The assay was terminated after 4 h by fixation of the cells with 2.5% glutaraldehyde in PBS⁶. The extent of morphological differentiation, expressed as the percentage of cells with neurites, was determined as described previously¹. A similar standard curve (not shown) is obtained with increasing amounts of conditioned medium from C6 glioma cells. One unit of glial factor activity is defined as the amount required to increase the extent of morphological differentiation by 1%. For comparative purposes, all activities are expressed in units per μg DNA in the corresponding primary culture.

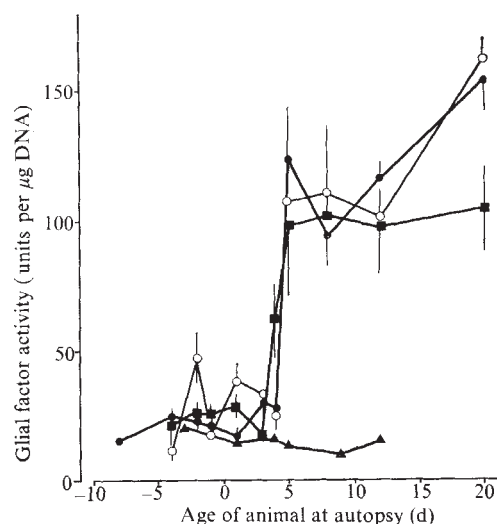


Fig. 2 GF-like activity in the medium conditioned by rat primary cultures initiated at different developmental stages. ●, Whole brain; ○, cerebral cortex; ■, cerebellum; ▲, kidney.

conditioned by kidney, lung, heart or liver primary cultures initiated during a similar perinatal period (results not shown).

Our results indicate that GF activity is not only a property of established cell lines⁴, but that a similar activity

Table 1 Glial factor-like activity in media conditioned by rat primary cultures

	5 days	Adult
Whole brain	124 ± 20	221 ± 39
Cerebral cortex	107 ± 10	271 ± 46
Cerebellum	99 ± 26	277 ± 30
Kidney	13 ± 1	
Lung	21 ± 2	
Heart	32 ± 2	
Liver	44 ± 2	
C6 glioma cells	99 ± 9	

Rats (Tif:RAIf (SPF)) were killed by decapitation and organs removed and cleaned of surrounding tissue in sterile conditions. The brain was dissected into cerebellum and cerebral cortex freed from the hippocampus. For practical reasons, dissociated cells from brain tissue of animals up to 8 d old were obtained by mechanical disruption through vigorous pipetting and passing through a 21 gauge needle. Dissociated cells from other tissues were obtained after enzymatic digestion³; fresh enzyme solution 4 × 10 min for brain tissues > 8 d old and 4 × 15 min for all other tissues. Cell suspensions were filtered through a 45-µm nylon mesh and plated at a suitable density (2–3 × 10⁶ viable cells per Petri dish) to give a confluent monolayer within 5–10 d. Such enzymatic treatment of brain tissue of animals up to 8 d old does not influence the appearance of GF like-activity in the corresponding cultures. All cells were grown in 2 ml of modified Leibowitz's medium on 35-mm plastic tissue culture dishes (Corning): 100 ml of medium containing 74 ml basal L-15 medium⁸, 13,000 U penicillin G, 2.6 mg streptomycin sulphate, 10% foetal calf serum (FCS) (Colorado Serum Co.; heat-inactivated for 30 min at 55 °C) and supplemented with glutamine, glucose, sodium bicarbonate to final concentrations of 1.73 mM, 22.1 mM and 100 mM, respectively. Culture dishes used for brain cells were precoated with poly-L-lysine⁴, which improves cell attachment without affecting the amount of GF-like activity. All cultures were maintained at 37 °C in a humidified 5% CO₂–95% air atmosphere. After the first 2 d, the cells were washed with Ca₂²⁺- and Mg²⁺-free phosphate-buffered saline (NaCl, 10 g l⁻¹; KCl, 0.25 g l⁻¹; Na₂HPO₄ · 2H₂O, 1.795 g l⁻¹; KH₂PO₄, 0.25 g l⁻¹), and the medium was renewed 2 or 3 times a week. Just before confluency, the medium was replaced with one containing 10% heat-inactivated horse serum (HS) (Microbiological Associates) and 48 h later the conditioned medium was collected, filtered (0.45 µm) and stored at –20 °C. For the determination of DNA content, cells were scraped off the plate in 0.5 ml of 0.5 M perchloric acid, sonicated and processed by the method of Burton⁵. The specific activities are expressed in units per µg DNA ± s.e.m. All activities were determined by testing two different aliquots of conditioned medium, each on three neuroblastoma test plates as described in Fig. 1.

is also produced by brain primary cultures. This observation gives more significance to a possible *in vivo* role for glial factor. For example, results with anatomical studies have suggested a major role for glial cells in reinnervation phenomena^{7,8}, and it is tempting to speculate that factors such as the one we describe may be involved.

There is a sharp rise in detectable activity in brain primary cultures derived from animals on days 3–5 after birth. This may reflect either an increase in the synthetic capacity of certain cells or an increase in the number of cells which are able to synthesise glial factor. The composition of the population of cells in the primary culture changes with the developmental stage at which the autopsy is performed. It is impossible to associate the increase of the GF-like activity with the morphological appearance of certain cell types in the culture. An answer to this question will require biochemical and/or immunological characterisation of the cell populations. It is tempting to correlate these results with the burst of glial cell multiplication starting at days 3–5 after birth^{9,10}. We suggest that the increase in GF-like activity reported here could be involved in the current development and neural arborisation taking place during this postnatal phase of rat brain maturation. Such a hypothesis requires the biochemical characterisation of the GF-like activity from rat brain primary cultures. Nevertheless, the fact that conditioned medium from primary cultures of kidney, lung, heart and liver do not show a similar increase in GF-like activity during postnatal development strongly suggests a specific role for GF in the development of the nervous system.

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Haloperidol-induced presynaptic dopamine supersensitivity is blocked by chronic lithium

SUPERSENSITIVITY following the interruption of synaptic transmission within the dopamine (DA) system has now been established by behavioural^{1–3}, biochemical^{4–6} and electrophysiological^{7,8} evidence. Following production of lesions which interrupt DA input¹, or treatment with pharmacological agents which chronically disrupt dopaminergic transmission^{3,9,10}, an increase in the sensitivity of post-synaptic (receiving input from dopaminergic neurones) responses of cells to DA and DA agonists has been observed. However, several studies have suggested that *in vivo*, pre-synaptic DA or 'autoreceptors' (that is, dopaminergic sites on DA-containing cells) may have an important physiological role in the modulation of DA synthesis and release^{11–13}. Thus, alterations in the sensitivity of pre-synaptic cells may have profound effects on the availability of the neurotransmitter. To date, only one study has attempted to investigate whether dopaminergic supersensitivity could be a presynaptic, as well as a postsynaptic,

phenomenon; following long-term treatment of rats with a phenothiazine, an increase in the sensitivity of DA-containing neurones in the substantia nigra to the DA agonist, apomorphine, has been observed¹⁴. We present here electrophysiological evidence for increased presynaptic sensitivity following chronic haloperidol treatment, as measured by both the direct iontophoretic application of DA and the systemic administration of apomorphine to DA-containing cells in the zona compacta region of the substantia nigra. In addition, it has been found recently that rats treated concurrently with lithium and haloperidol fail to develop post-synaptic supersensitivity, as measured by alterations in behavioural sensitivity^{15,16} and DA-receptor binding¹⁶. Several groups have theorised that alterations in catecholamine receptor sensitivity may be a factor in the aetiology of affective disorders, especially manic-depressive illness^{17,18}. As lithium therapy has been shown to be effective in preventing recurrent episodes of mania and depression in manic-depressive illness¹⁹, it is of interest to determine whether, at the level of individual DA neurones in the CNS, chronic lithium treatment could also affect the development of presynaptic supersensitivity. We provide here electrophysiological evidence for the blockade of presynaptic supersensitivity development following chronic lithium treatment.

Thirty-two male Sprague-Dawley rats (220–250 g) were used in these studies. Sixteen rats were provided with a diet for an ad libitum consumption consisting of 1,500 g of powdered laboratory (Purina) chow mixed with 2,000 cm³ of water and 2,266 mg of lithium carbonate (Li⁺). This lithium diet has been found to maintain lithium levels in rat serum at 0.8–1.0 mEq l⁻¹ (ref. 16), equivalent to therapeutically effective levels in humans. In this study, four rats similarly maintained on the Li⁺ diet for 14 d were found to have serum and brain tissue levels of Li⁺ of 0.8 mEq l⁻¹, as measured by atomic absorption spectrophotometry. The remaining 16 rats were placed on a similar diet, not containing lithium carbonate (CONT). Because of the marked polyuria observed in the Li⁺-treated animals, water and saline²⁰ were provided for ad libitum consumption by both groups, and the volume consumed was measured daily. On day 8, one-half of the Li⁺-fed and one-half of the CONT-fed rats were injected with 1 mg per kg of haloperidol (HAL) daily. The remaining half of each group received daily injections of saline (SAL). On day 29, daily HAL and SAL injections were terminated and all animals were placed on the CONT diet, containing no Li⁺.

Beginning on day 36, 7 d after the cessation of drug treatments, the single-unit activity of zona compacta cells in the substantia nigra was monitored following both the direct iontophoresis of DA and GABA, and the intravenous administration of graded doses of the DA agonist, apomorphine (APO). At least one animal from each treatment group (SAL-CONT, HAL-CONT, SAL-Li⁺ and HAL-Li⁺) was used during the recording session on each day until the entire series of recordings was completed on day 41, 12 d after cessation of drug treatments. Procedures for recording from identified DA cells were as described by Bunney *et al.*²¹. Specific details are contained in the legend to Fig. 1. Following the successive iontophoretic application of DA (10 nA for 60 s) and γ -aminobutyric acid (GABA) (5 nA for 60 s), and the return of cell firing to baseline rates, apomorphine was injected into the tail vein in exponentially increasing doses (from 1.2 to 80 μ g per kg) at 2-min intervals until complete inhibition of DA cell firing was achieved (Figs 1 and 2). Following the recording period, animals were killed, and blood and cerebral cortex were removed for Li⁺ determination. At the time of recording (7–12 d after cessation of the Li⁺ diet), Li⁺ levels were undetectable (<0.05 mEq l⁻¹) in both serum and brain tissue.

Iontophoresis of 10 nA of DA produced an average inhibition of 38% in the DA-cell firing rate in animals receiving

SAL injections daily on either control or Li⁺-containing diets (Fig. 1a and b, Table 1). Thus, chronic Li⁺ treatment alone did not apparently alter the sensitivity of the DA-cell to DA. This seems to differ from the effects of the direct application of Li⁺ (acute, applied by iontophoresis) which has been reported to antagonise the depressant response of another catecholamine, noradrenaline, in the hippocampus²². In addition, iontophoresis of 5 nA of GABA produced an average inhibition of 67% in the DA-cell firing in both CONT and Li⁺-fed rats, suggesting that GABA sensitivity was also not altered by chronic Li⁺ treatment. Iontophoretic

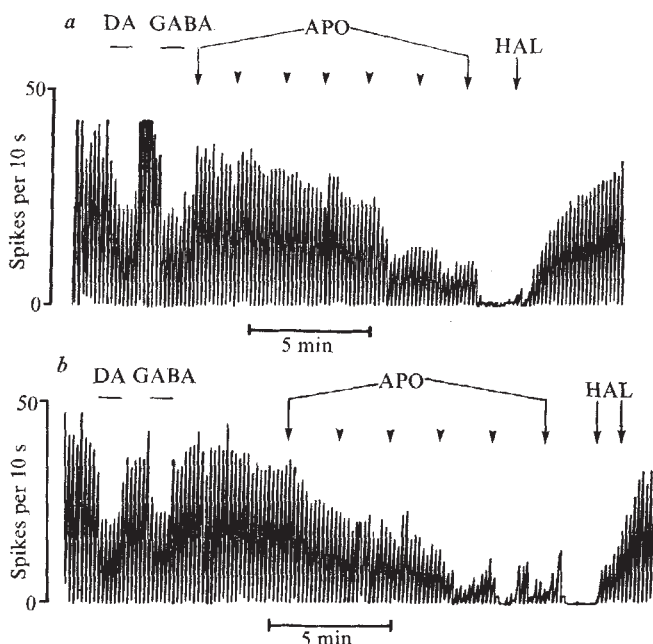


Fig. 1 DA-containing cells in the substantia nigra were tentatively identified by their wave form (a notched positive, then negative wave of long (approximately 2 ms) duration) and by their relatively slow, occasionally bursting spontaneous discharge rate (2–7 spikes s⁻¹). This firing pattern has been demonstrated by combined single-cell recording and histochemical fluorescent methods²¹ to be characteristic for DA-containing cells, but not for non-DA-containing cells in the adjacent zona reticulata of the substantia nigra or reticular formation. In these studies a five-barrel micropipette was prepared as previously described²⁸ and the central barrel was filled with 2 M sodium chloride saturated with fast green dye. Side barrels contained 0.5 M dopamine (pH 4.0) and 0.01 M GABA in 0.2 M sodium chloride (pH 4.0). At the end of the recording period, the recording site was marked by ejection of a spot of fast green dye. Brain tissue was fixed and examined histologically for localisation of the recording site. Localisation of the recording site within the A9 region was a necessary criterion for inclusion of data in this study. *a*, Saline-control. The activity of a single DA cell is plotted as a histogram of spikes per 10 s against time. The response of this DA cell to the microiontophoretic application of DA and GABA was measured in a rat fed a control diet receiving daily saline injections terminated 11 d before the recording session. DA, ejected with a 10 nA current for 60 s (bar), produced a 51% inhibition of activity, and GABA, ejected with 5 nA of current for 60 s (bar) produced a 55% inhibition of firing; apomorphine (APO, cumulative dose of 80 μ g per kg, 1.2, 1.2, 2.5, 5, 10, 20, 40 μ g per kg at arrows) produced a total inhibition of firing; haloperidol (HAL, 0.1 mg per kg intravenously at arrow) reversed this inhibition. *b*, Saline-lithium. The activity of a DA cell in a rat fed a diet containing lithium (Li⁺) and receiving daily saline injections terminated 8 d before the recording session is shown following the iontophoretic application of DA and GABA. DA, ejected with 10 nA of current for 60 s produced a 50% inhibition of activity, and GABA, ejected with 5 nA of current for 60 s produced a 42% inhibition of firing. APO (cumulative intravenous dose of 40 μ g per kg, 1.2, 1.2, 2.5, 5, 10 and 20 μ g per kg at arrows) produced a total inhibition of neuronal activity; haloperidol (0.1 mg per kg at arrows) reversed this inhibition.

Table 1 Effects of iontophoretically applied DA and GABA and systemic apomorphine on the firing of DA neurones in control lithium and/or chronically haloperidol-treated rats

Treatment* (no. of animals)	Effects on DA cell firing rates (mean $\pm$ s.e.m.)		
	% of baseline activity†		
	Microiontophoretically‡ applied DA (10 nA)	Microiontophoretically§ applied GABA (5 nA)	Cumulative intravenous dose (μ g per kg) of apomorphine to inhibit firing by 50% (ID ₅₀)
Saline-control (7)	37.4 $\pm$ 3.8	66.7 $\pm$ 11.6	22.5 $\pm$ 3.28
Saline-Li ⁺ (5)	39.2 $\pm$ 4.0	67.6 $\pm$ 12	17.5 $\pm$ 7.2
HAL-control (6)	67.2 $\pm$ 9.5	73.8 $\pm$ 9.0	3.75 $\pm$ 0.6
HAL-Li ⁺ (7)	46.8 $\pm$ 8.4	55.2 $\pm$ 10.3	17.1 $\pm$ 3.9

*See text for description of chronic drug treatments.

†The 10-s firing rate of the neurone, averaged over a 1-min period before the iontophoretic application of either 10 nA of DA or 5 nA of GABA, was compared to the 10-s firing rate averaged during the 60-s period of iontophoresis of DA or GABA.

‡One-way analysis of variance revealed significant main effects only for the iontophoretic DA responses ($P < 0.05$), while a *post hoc* comparison showed these differences to be due to the HAL-SAL DA responses. Thus, both the CONT-SAL against HAL-CONT ($F = 3.9$, d.f. = 20) and the HAL-CONT against HAL-Li⁺ ($F = 3.4$, d.f. = 20) comparisons were significantly different ($P < 0.05$), while all other comparisons were not significantly different.

§No significant differences were found for iontophoretic GABA responses for any drug treatments.

||One-way analysis of variance revealed significant differences in the dosages of APO required to inhibit respective treatment groups, while a *post hoc* comparison showed that these effects were due to the HAL-CONT APO responses. Thus, both the CONT-SAL against HAL-CONT ($F = 2.99$, d.f. = 17) and the HAL-CONT against HAL-Li⁺ ($F = 2.99$, d.f. = 17) comparisons were significantly different ($P < 0.05$), and all other comparisons failed to reach statistical significance.

application of Li⁺ (acute) has also been reported not to affect the sensitivity to GABA²².

However, as reported for postsynaptic striatal cells⁸, chronic HAL treatment in our experimental conditions produced a significant increase in the sensitivity of the presynaptic (DA-containing) cells to DA (an average inhibition of 67% from baseline firing rates) (Fig. 2a, Table 1). Iontophoretically applied GABA seemed to be equally effective in both the SAL (67% inhibition) and HAL-treated (74% inhibition) animals, suggesting that the increased sensitivity is somewhat selective to DA (Fig. 2a, Table 1).

In contrast, animals receiving both daily HAL injections and chronic Li⁺ treatment did not show a significant increase in sensitivity of the presynaptic receptors to iontophoretic DA, 47% inhibition as compared to 38% inhibition in non-HAL treated rats (Fig. 2b, Table 1). Again, no significant differences in GABA sensitivity were observed (Fig. 2b, Table 1).

Because an important criterion of supersensitivity⁴³ is a shift to the left in the dose-response relationship to an exogenous transmitter or agonist, we also evaluated the effects of graded intravenous doses of the dopamine agonist, apomorphine (Merck), on the firing rate of DA-containing (presynaptic) cells. As reported previously, apomorphine depresses the neuronal activity of DA cells^{21,24}, and this depression is reversed by anti-psychotic drugs such as HAL. As can be seen in Fig. 3, a shift in the dose-response curve in the direction of increased sensitivity was observed for animals receiving a daily HAL injection but maintained on a control diet. In contrast, animals receiving daily HAL injections but maintained on a diet containing Li⁺ did not show this altered sensitivity to apomorphine.

Thus, these data provide direct evidence that the sensitivity of presynaptic DA cells, like that of postsynaptic DA cells, can be altered by chronic treatment with haloperidol. In addition, chronic Li⁺ treatment apparently prevents this increased sensitivity of presynaptic DA cells. Although blockade of the development of supersensitivity is a likely explanation for our findings, the increased polyurea observed in Li⁺-treated animals could result in a faster elimination of HAL from the brain. However, using the same drug treatment schedules, no significant differences in brain levels of [³H]HAL were observed between chronic HAL- and HAL-Li⁺-treated rats measured over a 24 h period following the final HAL injection (mean 24-h [³H]HAL level in chronic HAL animals = $6.88 \pm 0.28 \mu$ g per g, mean 24-h [³H]HAL level in HAL-Li⁺ animals =

$6.53 \pm 0.25 \mu$ g per g, $P > 0.05$ (ref. 16 and J. Rosenblatt, personal communication).

The mechanism for the development of and the physiological significance of presynaptic supersensitivity is not yet understood^{14,25}. However, the finding that the therapeutically

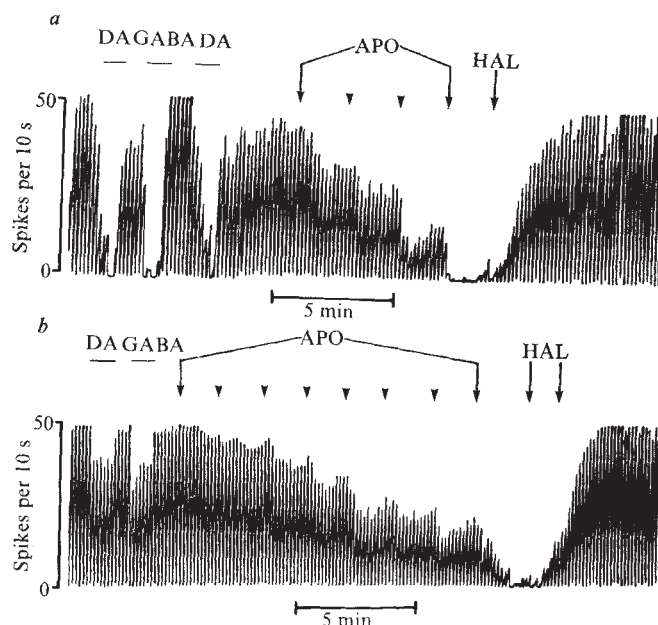


Fig. 2 Cells recorded and identified as in Fig. 1. *a*, Haloperidol-control. DA cell activity in a rat receiving a control diet and daily 1 mg per kg intraperitoneal injections of haloperidol (HAL) terminated 8 d before the recording session. Iontophoresis of 10 nA of DA for 60 s (bar) produced a 100% inhibition of neuronal activity, and ejection of 5 nA of GABA for 60 s (bar) also produced complete inhibition of firing. A 10 μ g per kg cumulative dose of apomorphine (APO, 1.2, 1.2, 2.5 and 5 μ g per kg at arrows) was sufficient to inhibit DA cell firing totally; HAL (0.1 mg per kg at arrow) reversed this inhibition. *b*, Haloperidol-lithium. The activity of a DA cell is recorded in a rat which had received a diet containing lithium (Li⁺) and daily injections of HAL (1 mg per kg, intraperitoneally). Treatment was terminated 8 d before the recording session. Iontophoresis of 10 nA of DA for 60 s produced a 20% inhibition of firing; ejection of 5 nA of GABA for 60 s produced a 30% inhibition of neuronal activity in this cell. The intravenous administration of APO (cumulative dose at 160 μ g per kg, 1.2, 1.2, 2.5, 5, 10, 20, 40 and 80 μ g per kg at arrows) produced an inhibition of firing; HAL (0.1 mg per kg at arrows) reversed this inhibition.

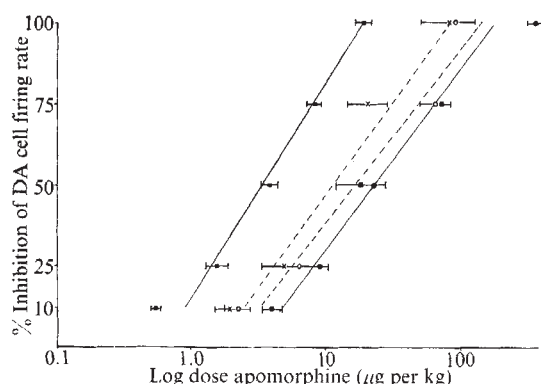


Fig. 3 Effects of graded doses of apomorphine (APO) on DA cell activity in animals after various chronic drug treatments (as described in text). Beginning 7 d after the cessation of chronic drug treatments, rats from each treatment group were anaesthetised with chloral hydrate (400 mg per kg, intraperitoneally) and prepared for recording. The single-unit activity of zona compacta cells in the substantia nigra (see legend Fig. 1) was monitored while APO was administered intravenously in exponentially increasing doses from 1.2 µg per kg to 80 µg per kg at 2-min intervals or until a total inhibition of DA cell firing was achieved. The ordinate indicates the reduction in DA neuronal activity and the abscissa the dose of APO required to achieve this reduction. Lines have been plotted using linear regression analysis, and data points represent the mean values of at least 4 animals. Horizontal bars depict s.e.m. for dose of APO at the specified level of inhibition. A factorial repeated-measures analysis of variance revealed significant main effects for both variables ($F=8.72$, d.f. = 3/17, $P < 0.01$ for drug treatments and $F=24.2$, d.f. = 4/68, $P < 0.01$ for % inhibition). A nonsignificant interactive effect ($F=1.4$, d.f. = 12/68, $P > 0.05$) indicates that the dose-response functions for all drug treatments are parallel. A *post hoc* comparison between haloperidol-saline and the other three drug treatments was found to be significant ($P < 0.01$). ■, Haloperidol-control; ×, saline-Li⁺; ○, haloperidol-Li⁺; ●, saline-control.

effective agent, lithium, can block this process suggests that the phenomenon of supersensitivity development in both pre- and postsynaptic neurones may have clinical relevance. Further experiments are in progress to determine whether chronic lithium treatment may also affect the sensitivity of pre- and postsynaptic neurones in other CNS transmitter systems.

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Inactivation without facilitation of calcium conductance in caesium-loaded neurones of *Aplysia*

THE concentration of cytoplasmic free calcium (Ca) ion regulates a variety of cellular processes¹⁻⁴, including hormone secretion, histamine secretion, neurotransmitter release, mitosis and muscle contraction. For some of these processes the activating signal is a rapid influx of Ca ions across the plasma membrane during an action potential. One way of controlling the magnitude of such processes would be a regulation of the amount of Ca influx during each action potential. A mechanism capable of this type of control has recently been ascribed to the Ca conductance system of molluscan neurones. It is called 'Ca facilitation', and is an increase in the inward Ca current (I_{Ca}) observed when a test voltage-clamp pulse is preceded by a depolarised, conditioning prepulse⁵⁻⁸. Ca facilitation could cause an enhanced Ca influx during a repetitive train of action potentials. An opposing process, Ca inactivation, has also been described in excitable tissue⁹⁻¹³. Ca inactivation, like Na inactivation, is a decrease in inward current caused by a depolarising prepulse. Thus, facilitation would cause an increase and inactivation a decrease in I_{Ca} during repetitive voltage-clamp pulses. Facilitation of I_{Ca} has not been observed directly under voltage-clamp because inward I_{Ca} is usually accompanied by a large, outward K current (I_K) (ref. 14). We have eliminated I_K by using a new technique to replace intracellular potassium with caesium, and report here that I_{Ca} inactivates without evidence of facilitation.

Voltage-clamp records were obtained from the neurones R2 and R15 in the visceral ganglion of *Aplysia californica*. The neurone of choice was impaled with two micro-electrodes (filled with 3 M CsCl or 3 M KCl, see below). The general experimental procedure and voltage-clamp circuit were described previously⁷. A step voltage-clamp had a rise time of 100 µs. To observe I_{Ca} in the absence of I_K , Cs was substituted for intracellular K using the antibiotic nystatin, which makes the cell membrane of *Aplysia* neurones highly permeable to monovalent cations¹⁵. Na-free solutions in the bath eliminated both I_{Na} and artefacts in clamp records due to uncontrolled regions on the axon. The bath temperature was maintained at 14-16 °C for all experiments.

Cs-loading was accomplished by bathing the ganglion in a Cs-loading solution (see Table 1) enriched with 40 mg l⁻¹ nystatin (Sigma) for 10-15 min. During this period the resting potential was 0 ± 5 mV, and the membrane conductance was unmeasurably large. This procedure reduces the intracellular K concentration from about 160 mM to less than 30 mM, by Cs substitution¹⁵. The nystatin was then washed out of the membrane with a nystatin-free, Cs-loading solution. After 30 min in this solution the resting conductance returned to a normal level (about $5 \times 10^{-7} \Omega^{-1}$ for R2 or $5 \times 10^{-6} \Omega^{-1}$ for R15), and the cell was voltage-clamped to a holding potential (V_h) of -40 mV. The bath was then replaced with normal saline (Table 1). To prevent intracellular K contamination, 3 M CsCl-filled micro-electrodes were used, and all test solutions were K-free. As a control for the effects of nystatin, K-loading was carried

out on six neurones impaled with KCl-filled microelectrodes. These neurones were indistinguishable from intact neurones under voltage-clamp conditions.

Figure 1 shows net currents recorded under voltage-clamp in a K-loaded and a Cs-loaded neurone. Figure 1c shows early inward and late outward current in a K-loaded cell bathed in 0 Na/100 Ca (Table 1). Figure 1d shows the abolition of the inward current and a reduction in outward current by the replacement of Ca by 20 mM Co^{2+} . The reduction in outward current may be due, in part, to the absence of Ca-activated I_K in this solution^{16,17}. Figure 1a shows a prolonged inward current in a Cs-loaded neurone bathed in 0 Na/100 Ca. Similar currents have been observed in snail somata internally perfused with K-free solutions containing Tris or Cs^{11,13}. The inward current in Fig. 1a is likely to be carried by Ca ions, as the replacement of Ca by Co abolishes the inward current (Fig. 1b). The small, time-invariant, outward current observed in Fig. 1b may be a nonspecific leakage current, and suggests that Cs is impermeable in the rectifying K channel. Cs is probably also permeable in the Ca-activated K channel since in snail neurones internally perfused with Cs, the instantaneous current-voltage relationship of I_{Ca} is approximately linear and extrapolates to a value close to that of the Ca equilibrium potential¹³.

Figure 2 shows a double-pulse experiment. Identical 50-ms voltage pulses were separated by a 200-ms interpulse interval; previous studies reported this to be an optimal interval for facilitation of I_{Ca} (refs 6–8). Figure 2a shows that outward current decreased in a K-loaded cell during repetitive stimulation to +30 mV. Decreases in I_K during repetitive stimulation have been observed previously in molluscan neurones^{5–8,18,19}. Figure 2b shows that I_{Ca} also decreased during repetitive stimulation of a Cs-loaded neurone. The decrease is apparent both in the early peak and in the later, prolonged phase of I_{Ca} . Figure 2c plots the current-voltage relationship for the maximum inward current of two identical voltage pulses in a Cs-loaded neurone. At all potentials I_{Ca} was greater during the first pulse. The currents of both pulses (PI and PII) peaked at about +30 mV. The greatest inactivation occurred from +20 to +40 mV, also peaking around +30 mV. The dashed line in Fig. 2c is a linear extrapolation of hyperpolarising leakage points. The intersection of this leakage line with measurements of peak current at positive potentials is probably due to a non-linearity of the leakage conductance rather than to a reversal of I_{Ca} .

The decrease in net inward current during repetitive clamp pulses may be caused by a decrease in inward I_{Ca} , or an increase in outward current. In order to test for the latter possibility we examined Cs-loaded neurones in 0 Na/20 Co. No change in the small 'leakage' current was

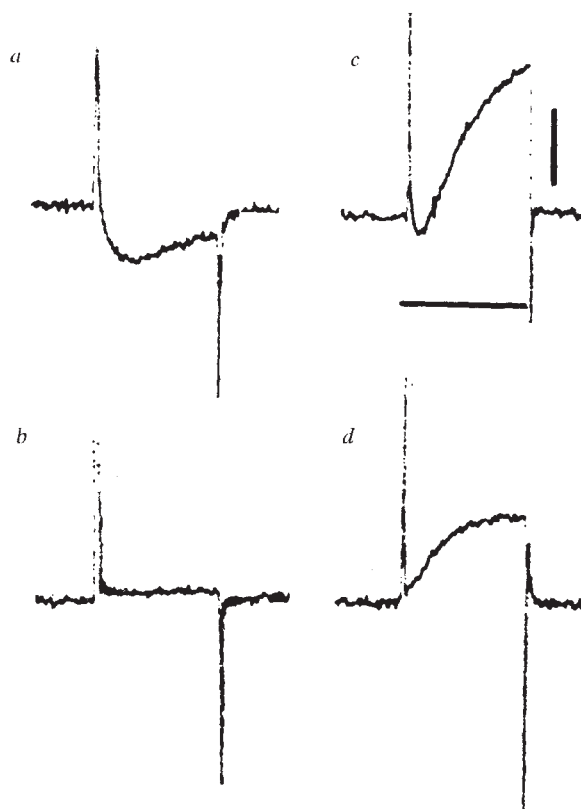


Fig. 1 Net soma current measured with a two electrode voltage-clamp. In all traces, inward (negative) current is shown as a downward deflection. A caesium-loaded R15 cell with a holding potential (V_h) of -42 mV and clamped with a 50-ms pulse to +27 mV in 0 Na/100 Ca (a) and 0 Na/20 Co (b). A potassium-loaded R15 with V_h = -45 mV, clamped with a 50-ms pulse to +26 mV in 0 Na/100 Ca (c) and 0 Na/20 Co (d).

observed during repetitive pulses to +30 mV, supporting the notion that the decrease in net inward current observed in the presence of Ca is due to a decrease in inward I_{Ca} during repetitive pulses.

Figure 3 shows the effect of interpulse interval on I_{Ca} in a double-pulse experiment; 50-ms voltage pulses to +24 mV were separated by a variable interval. The inset of Fig. 3 shows the decrease in I_{Ca} at interpulse intervals of 200 ms, and 1 and 8 s. At all interpulse intervals ranging from 20 ms to 10 s, the peak inward I_{Ca} was smaller during the second pulse. The plot of Fig. 3 is the relative magnitude of peak I_{Ca} during pulse 2 (I_2/I_1) against the interpulse interval. The relative magnitude of I_{Ca} was directly related to interpulse interval. The dashed line in Fig. 3 is the sum of two exponential functions with time constants of 420 ms and 4.4 s. In another Cs-loaded neurone (not shown), the two time constants for Ca inactivation at this membrane potential were 450 ms and 4.7 s. Kostyuk *et al.* have also observed two time constants for Ca inactivation in perfused snail neurones¹¹. We have examined eight Cs-loaded neurones, and have never observed an increase in I_{Ca} during repetitive stimulation within the voltage and interval ranges of Figs 2 and 3.

In previous studies, the existence of a facilitation of I_{Ca} has not been observed directly. In this report we have examined I_{Ca} directly in Cs-loaded cells in the absence of extracellular Na. We have not examined the possibility that extracellular Na or intracellular K are necessary for Ca facilitation, because the presence of these cations usually results in currents that obscure I_{Ca} . The possibility also exists that Cs abolishes Ca facilitation. Another K current blocker, tetraethylammonium, has been postulated to block Ca facilitation in snail neurones¹⁷. However, it seems un-

Table 1 Composition of solutions (mM)

	Loading solutions					Nystatin
	KCl	CsCl	Sucrose	MgSO ₄	Tris*	
Cs loading	0	300	394	100	10	†
K loading	300	0	394	100	10	†
	Test solutions					Tris*
	NaCl	KCl	CaCl ₂	MgCl ₂	CoCl ₂	
Normal saline	494	‡	5	50	0	30
0 Na/100 Ca	0	‡	100	50	0	410
0 Na/20 Co	0	‡	0	50	20	560

*Tris (hydroxymethyl) aminomethane (Sigma) was titrated to pH 7.7 with HCl.

†Nystatin was dissolved in methanol (5 mg ml⁻¹) and could subsequently be added to a loading solution at a final concentration of 40 mg l⁻¹.

‡In K-loaded cells, KCl was 10 mM in all test solutions. In Cs-loaded cells KCl was omitted from test solutions.

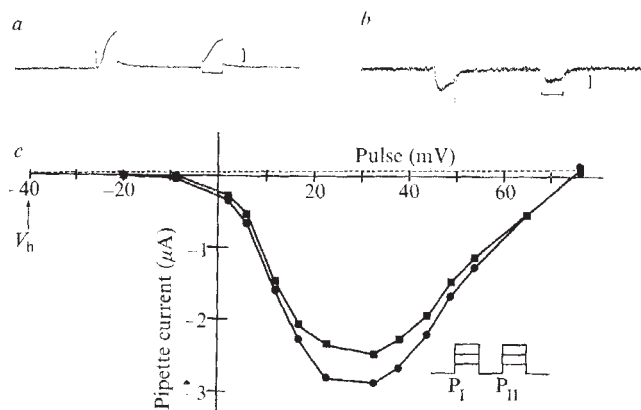


Fig. 2 The effect of prior depolarisation on currents of potassium-loaded (*a*) and caesium-loaded (*b*) cells. *a* Is a K-loaded R15 with $V_h = -36$ mV and clamped with identical 50-ms pulses to $+36$ mV (horizontal bar = 50 ms, vertical bar = $1 \mu\text{A}$). *b* Is a Cs-loaded R15 held at -35 mV and clamped to $+35$ mV (horizontal bar = 50 ms, vertical bar = $0.5 \mu\text{A}$). The interpulse interval for both *a* and *b* was 200 ms. *c* Shows the voltage dependence of inactivation of peak calcium current (I_{Ca}). A Cs-loaded R2 cell with $V_h = -40$ mV was clamped with two identical pulses (PI (●) and PII (■); 50-ms duration, 200-ms interpulse interval). The amplitude of the pulses was varied and peak current of each pulse is plotted as a function of amplitude. The dashed line is a linear extrapolation of the leakage, measured at potentials more negative than V_h .

likely to us that the presence or absence of cations such as Na, K and Cs would have the capacity to change a facilitating conductance into an inactivating conductance. Such pharmacological effects of these cations have not been described in the literature, and therefore must remain speculative until demonstrated. The absence of facilitation of I_{Ca} cannot be explained by the high concentration (100 mM) of Ca used in the above experiments, as similar results were also obtained in 10 to 20 mM Ca concentrations in three cells.

Ca influx during an action potential is not controlled entirely by the properties of Ca conductance. Action potentials of molluscan neurones increase in duration during repetitive firing, primarily due to a progressive reduction in I_K (refs 18,19). The increase in spike duration provides an increase in the time during which the Ca conductance remains activated by depolarisation. Although Ca conductance inactivates, the Ca influx per action potential may increase during repetitive firing, due to an increase in spike

duration. Such increases in spike duration have been observed at presynaptic terminals during repetitive firing²⁰⁻²². As synaptic facilitation is due to an increased release of neural transmitter from presynaptic terminals during repetitive firing²³, concomitant increases in Ca influx at these locations may have a role in synaptic facilitation²⁴.

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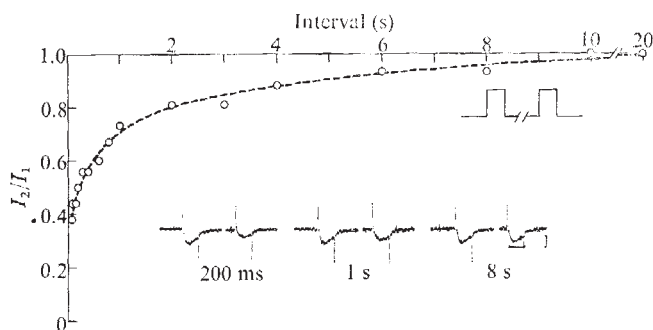
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Halobacterium strain 5 contains a plasmid which is correlated with the presence of gas vacuoles

GAS vacuoles are subcellular inclusions in certain prokaryotic organisms which enclose a gas-filled space and consist of a proteinaceous membrane containing one or two very hydrophobic protein subunits¹. Gas vacuoles are, with few exceptions, limited to aquatic microorganisms², where it has been proposed either that they may be involved in regulating buoyancy, or that the light-scattering properties of the inclusions may protect the cells from the deleterious effects of high light intensities³. Several species of *Halobacterium* possess gas vacuoles³, and in certain strains, forms which lack gas vacuoles arise at a relatively high frequency. The rate of loss of gas vacuoles in *Halobacterium* strain 5 occurs so often that regular recloning of individual colonies containing gas vacuoles is necessary to maintain the wild-type strain⁴. Gas vacuoles are also lost spontaneously in other aquatic microorganisms, and it was this observation which led Walsby to propose that the gas-vacuole protein might be carried on a plasmid⁵. Plasmids have not previously been shown to exist in *Halobacterium*. All the obligate halophiles as yet examined contain a satellite DNA which may account for between 11% and 36% of the total DNA (ref. 6, 7). Moore and McCarthy⁷, however, argued that the satellite DNA is not composed of multiple copies of a plasmid because its amount is unchanged by growing the cells in the presence of known plasmid-curing agents, and because total *Halobacterium* DNA renatures at a rate expected for single-copy DNA. Here I show that the wild-type *Halobacterium* strain 5 contains three plasmids, and also show a correlation between

Fig. 3 The effect of interpulse interval on inactivation of calcium conductance. A caesium-loaded R15 cell with $V_h = -36$ mV was clamped with identical 50-ms pulses (PI and PII) to $+25$ mV separated by a variable interval. The relative magnitude of peak I_{Ca} (I_2/I_1) is plotted against interpulse interval. The dashed line is the sum of two exponential functions with time constants of 420 ms and 4.4 s. Inset shows records at three intervals, 200 ms, and 1 and 8 s from the same cell as the plot. (Horizontal bar = 50 ms, vertical bar = $0.5 \mu\text{A}$.)



the presence of a plasmid of molecular weight (MW) 44×10^6 and the presence of gas vacuoles.

Halobacterium salinarum strain 5 was grown in the medium of Larsen⁴ as liquid or on plates solidified with 1.8% agar. Liquid shake cultures were grown at 42 °C and the exponential doubling time was 4.0 h. Plated cells were incubated at 42 °C and the plating efficiency was found to be greater than 98%. *Halobacterium* 5 produces gas vacuoles in the early stationary phase of growth (unpublished data) and it takes up to a week to be able to distinguish clearly between plated colonies with and without gas vacuoles. Colonies containing gas vacuoles were creamy pink in colour, whereas those without were a clear orange colour and had an appearance similar to colonies of cells containing gas vacuoles which had been placed briefly under high nitrogen pressure to collapse the vacuoles.

On average, 1% of colonies grown from individual cells of wild-type *Halobacterium* strain 5 lose gas vacuoles. This frequency of gas vacuole loss remained constant during the serial subcloning of gas-vacuolate colonies. Five independent isolates of *Halobacterium* strain 5 lacking gas vacuoles were picked and cultured. In no case do colonies containing gas vacuoles appear in cultures grown from colonies lacking gas vacuoles. The rate of reversion of gas-vacuolate-less lines is $<10^{-6}$ and no gas-vacuolate colonies were found even when cell samples were taken from the top of stationary phase standing cultures; a place where vacuole-containing cells would be expected to congregate. In addition, treatment of strains lacking gas vacuoles with mutagenic agents such as 2-aminopurine nitrate⁸, mitomycin C, and ultraviolet light (unpublished) did not result in the appearance of colonies containing gas vacuoles.

The plasmid content of *Halobacterium* was determined by analysing cell extracts prepared by the cleared lysate technique of Clewell and Helinski⁹. The lysozyme pretreatment step was omitted, and cells were lysed in the presence of the detergents Brij-58 and sodium deoxycholate in conditions which enable most of the chromosomal DNA to be removed from solution by centrifugation. The cleared lysate was treated with both pancreatic and T₁ ribonuclease, and then extracted with phenol. The extrachromosomal DNA in the lysate was concentrated by ethanol precipitation and individual DNA types present were separated by Agarose gel electrophoresis using the procedure of Meyers *et al.*¹⁰. Figure 1 shows an Agarose slab gel of DNA isolated from wild-type *Halobacterium* strain 5 and two gas vacuole-less isolates (7702; 7705). In the extract from the wild-type *Halobacterium*, four DNA-containing bands are present. Band A represents unresolved chromosomal fragments probably in the form of linear duplex DNA since similar bands are found in cleared lysates from prokaryotes without plasmids¹⁰, and the mobility of the band is unaffected by treatments which are known to radically change the conformation (and hence the mobility) of covalently closed circles (ccc) of DNA (Fig. 2). Bands B, C, D are extrachromosomal DNA in the form of right-handed supertwisted ccc-DNA. This identification is based on the following evidence. (1) Intense illumination of the cleared lysates in the presence of the intercalating dye ethidium bromide causes a discontinuous decrease in the mobility of the bands. This treatment is known to nick ccc-DNA¹¹, producing the open circular (oc) form of DNA which would migrate more slowly than the ccc form during electrophoresis¹². (2) Electrophoresis of cleared lysates in the presence of increasing concentrations of ethidium bromide results in an initial decrease in electrophoretic mobility of bands B, C, D followed by a return to greater mobility (Fig. 2). These results are expected for right-handed supertwisted DNA. When ethidium bromide intercalates ccc-DNA, it initially removes superhelical turns¹³. As the DNA is unwound, its electrophoretic mobility is decreased proportionally, and at some critical concentration, the ccc-DNA takes the same

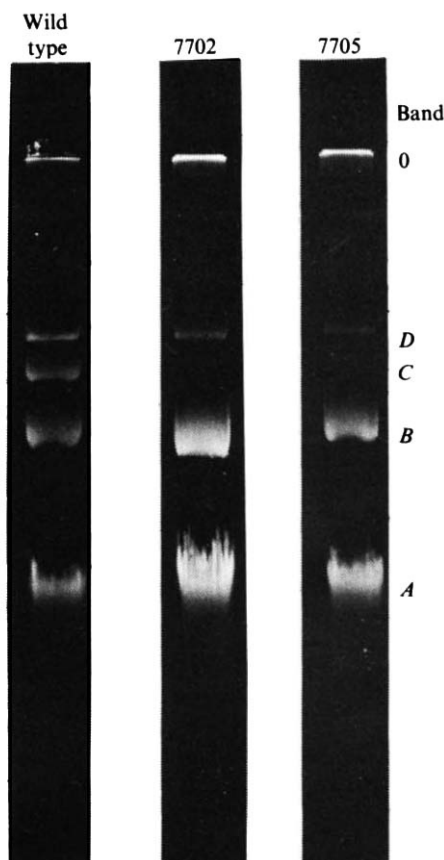


Fig. 1 Electrophoretic separation of DNA in cleared lysates from wild-type *Halobacterium* strain 5 and from two independent clones lacking gas vacuoles (7702, 7705). Equal numbers of cells were lysed using the procedure of Clewell and Helinski⁹ with the exception that pretreatment with lysozyme was omitted. Extracts were prepared, and identical amounts of cleared lysate were electrophoresed on 11.5 cm $\times$ 13 cm horizontal slabs of 0.5% Agarose as described by Meyers *et al.*¹⁰. The gel buffer and running buffer were composed of: 0.089 M Tris (hydroxymethyl) aminomethane, 0.089 M boric acid, and 0.0025 M EDTA. Electrophoresis was carried out at 2.9 V cm^{-1} at room temperature for 16 h. To identify the DNA, the gel was soaked in 1 $\mu\text{g ml}^{-1}$ ethidium bromide for 1 h and then illuminated with short wavelength ultraviolet light. In these conditions, DNA fluoresced a bright orange colour, and the position of the bands on each gel was recorded on Polaroid type 57 positive/negative film. 0 indicates the starting origin for electrophoresis.

form and mobility as oc-DNA. As the ethidium bromide concentration is further increased, the ccc-DNA becomes a left-handed supertwisted molecule with an electrophoretic mobility similar to the original right-handed supertwisted molecule¹⁴. The position of the minimum mobility for the plasmid species B, C, D in Fig. 2 is in agreement with the expectation that the larger the molecular weight of the DNA, the higher the concentration of ethidium bromide required to fully unwind the supertwisted DNA. Since band A has a greater mobility than band B, if the DNA in band A were in the form of ccc-DNA, the position of band A would have decreased to a minimum at a concentration of less than 0.10 $\mu\text{g ml}^{-1}$ ethidium bromide.

Because the plasmids found in *Halobacterium* strain 5 are ccc-DNA, their molecular weight can be determined by comparing their electrophoretic mobilities with that of a set of plasmids of known molecular weight (Fig. 3) (ref. 10). The molecular weights of the three plasmids found in the wild-type *Halobacterium* strain ($\pm 10\%$) are: 26×10^6 ; 44×10^6 ; and 86×10^6 .

In clones of *Halobacterium* strain 5 which have been isolated on the basis of the absence of gas vacuoles, the 26×10^6 and 86×10^6 MW plasmids are present, but the

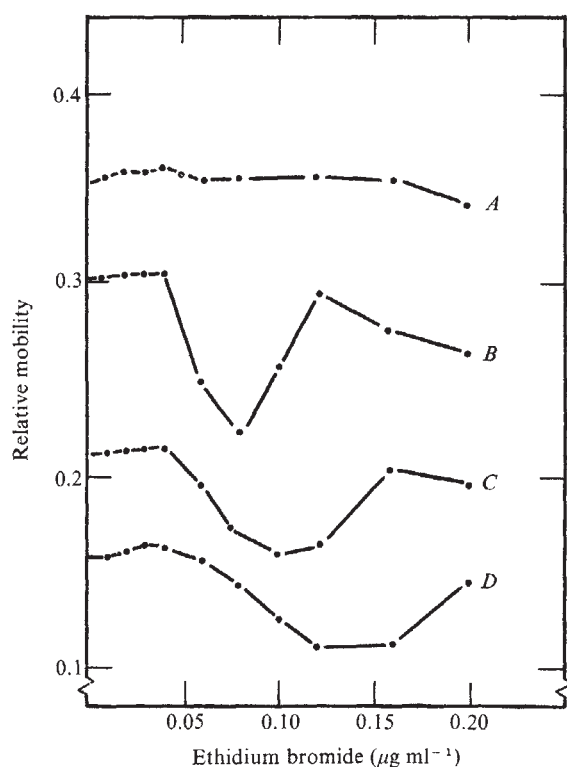


Fig. 2 Relative electrophoretic mobility of DNA-containing material in cleared lysates of wild-type *Halobacterium* strain 5 as a function of the ethidium bromide concentration present during electrophoresis. Samples were prepared and electrophoresed as in Fig. 1 with the exception that cylinders of 0.5% Agarose containing varying amounts of ethidium bromide were used as a support. The method of Espejo and Lebowitz¹⁴ was used to ensure a constant concentration of ethidium bromide in each gel tube during electrophoresis. Samples were run at 1 mA per tube for 4.0 h. The position of ethidium-staining bands is given relative to the position of a bromophenol blue dye marker and only main bands are listed. Individual bands have wide distribution at ethidium bromide concentrations which contain molecular species intermediate to either fully supercoiled forms or open circular forms, and in such cases, the average position of the band is given.

44×10^6 MW plasmid species is missing (Fig. 1). Five randomly picked clones of *Halobacterium* lacking gas vacuoles have been examined and in all cases the intermediate sized plasmid was absent. It is unlikely that the 44×10^6 MW plasmid DNA is present in a form other than ccc-DNA in gas vacuole-less isolates (as, for example, oc-DNA or integrated with the host chromosome) since no treatment of gas vacuole-less cultures ever resulted in the production of cells with gas vacuoles. In addition, the 44×10^6 MW plasmid is probably not lost because of the action of nonspecific nucleases since *Halobacterium* plasmids both larger and smaller in size remain unaffected. These results strongly suggest that the 44×10^6 plasmid is required for the expression of gas vacuoles.

It is possible that the satellite DNA previously reported in *Halobacterium* consists of plasmid DNA. Plasmid-curing agents have no effect on the rate of loss of gas vacuoles in *Halobacterium* (Table 1) and therefore such agents would not be expected to change the amount of satellite DNA. The ineffectiveness of these agents may be due to either the unusual properties of *Halobacterium* and the medium it grows in, or the plasmids may belong to a class which are not sensitive to the curing agents. *Halobacterium* strain 1 has a satellite DNA which amounts to 20% of the total DNA, and by renaturation kinetics, the total complexity of the DNA is estimated as 2.72×10^9 MW. Three copies per genome of the plasmids reported here would

comprise 0.47×10^9 , or 17.3% of the total DNA. Since it is not possible by optical renaturation methods to distinguish between one copy per cell and three copies per cell¹⁵, the satellite could easily be composed of the sum of the plasmid DNAs.

This is the first reported case of the involvement of a plasmid in the expression of a subcellular inclusion in a prokaryote. Identification of a plasmid as a vehicle for gas-vacuole expression may help to explain the evolutionary relationships between aquatic microbes which contain gas vacuoles. In this context, it would be of interest to examine the involvement of plasmids with gas vacuoles in other aquatic microorganisms; especially in light of the fact that spontaneous gas vacuole-less mutants of the aquatic aerobe *Prosthecomicrobium pneumaticum*¹⁶, as well as of several species of cyanobacteria^{5,17}, have already been described.

Gas vacuoles isolated from *Halobacterium* strain 5 have been shown to contain two protein components, one having a MW of 12,900 and representing 20% of the total protein and a second of 15,100 MW, which constitutes the remaining 80% (ref. 18). The amino acid composition of the total *Halobacterium* gas vacuole protein is similar to that of the protein isolated from the cyanobacteria³. It is possible that the plasmid of intermediate molecular weight codes for the gas vacuole proteins since $28,000$ MW of protein ($12,900 + 15,100$) would only represent the coding equivalent of 0.56×10^6 MW double-stranded DNA (less than 1.3% of the 44×10^6 MW plasmid). It is interesting to note that a spontaneous gas vacuole-less isolate of the cyanobacterium *Anabaena flos-aquae* was found to have lost the ability to synthesise the gas vacuole protein subunit⁵. The actual presence of the coding sequence for the gas vacuole protein on the 44×10^6 MW plasmid in *Halobacterium* can only

Fig. 3 Relative electrophoretic mobility of plasmids from *Halobacterium* strain 5 and plasmids of known molecular weight isolated from strains of *E. coli*. Cleared lysates of all bacteria were prepared and electrophoresed as in Fig. 1 with the exception that *E. coli* strains were pretreated with lysozyme at 0°C before lysis. The plasmids of known molecular weight used and their *E. coli* strains included: pSC101/C600, 5.8×10^6 ; pMB9/HB129, 7.3×10^6 (dimer)¹⁹; Sa/J53, 25×10^6 ; RK2/J53 and RP4/J53, 40×10^6 ; and R64/J53, 72×10^6 . The mobility of all plasmids listed are given relative to that of pSC101. The positions of *Halobacterium* strain 5 bands B, C, D (either wild type or without gas vacuoles) are indicated by the appropriate lettered arrows.

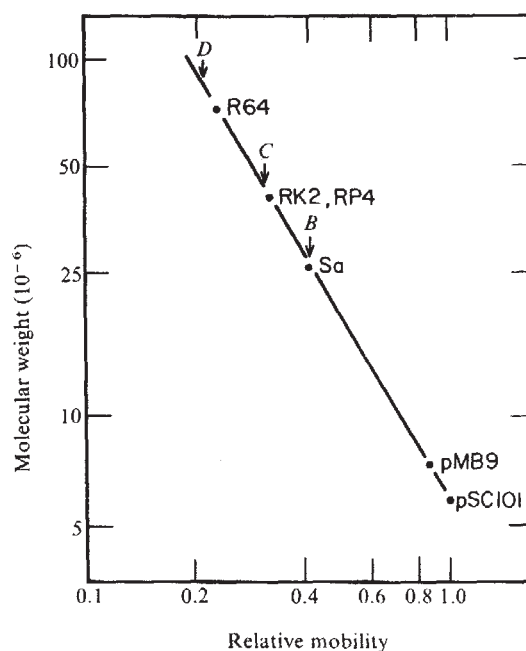


Table 1 Effect of curing agents on loss of gas vacuoles in *Halobacterium* strain 5

Compound	Minimal inhibitory concentration ($\mu\text{g ml}^{-1}$)	concentration ($\mu\text{g ml}^{-1}$)	% Colonies without gas vacuoles
Control	—	—	0.97
Ethidium bromide	2.5	1.0	1.47
Acridine orange	None, 200 is saturating	200	1.09
Neutral acriflavine	100	50	0.68
Sodium dodecyl	None, 100 is saturating	100	0.96

Cell cultures containing various amounts of plasmid curing agents were inoculated with wild-type *Halobacterium* strain 5 at a density of 10^5 cells ml^{-1} . Cultures were grown at 42°C and were aerated vigorously by shaking, to avoid selective enrichment for gas vacuolate cells¹⁶. The minimal inhibitory concentration is the lowest concentration which completely inhibited growth. Cell samples were plated when cultures had grown to 2×10^8 – 8×10^8 cells ml^{-1} . Following one week of growth in the dark, plates were scored for percentage of colonies without gas vacuoles as indicated in the text.

be demonstrated, however, either by mobilising the plasmid into a prokaryote not known to form gas vacuoles, or by isolating a mutant plasmid which specifies a mutant gas vacuole protein.

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The length of nucleosome-associated DNA is the same in both transcribed and nontranscribed regions of chromatin

THE fundamental structural unit of chromatin is a nucleoprotein complex containing two molecules each of the histones H2A, H2B, H3 and H4 in association with approximately 200 base pairs of DNA (for review see ref. 1). At least 85% of nuclear DNA is organised in these chromatin subunits or nucleosomes², and it has been shown that both transcribed^{3–8} and nontranscribed^{9–12} DNA sequences are arranged in nucleosomes. The length of DNA in the nucleosome repeat unit varies between organisms and even between cell types in the same organism¹. The origin

of this variation in repeat length, ranging from 160 to 240 base pairs per nucleosome, is unknown. However, there is a general correlation between repeat lengths and transcriptional activity: transcriptionally active cells have shorter nucleosome repeat lengths than less active cells¹³. Thus, it has been suggested that the nucleosome repeat length of transcribed DNA may be different from that of nontranscribed sequences, thereby implicating nucleosome organisation in gene regulation¹⁴. We report here that both transcribed and nontranscribed DNA sequences are organised in nucleosomes with the same repeat lengths.

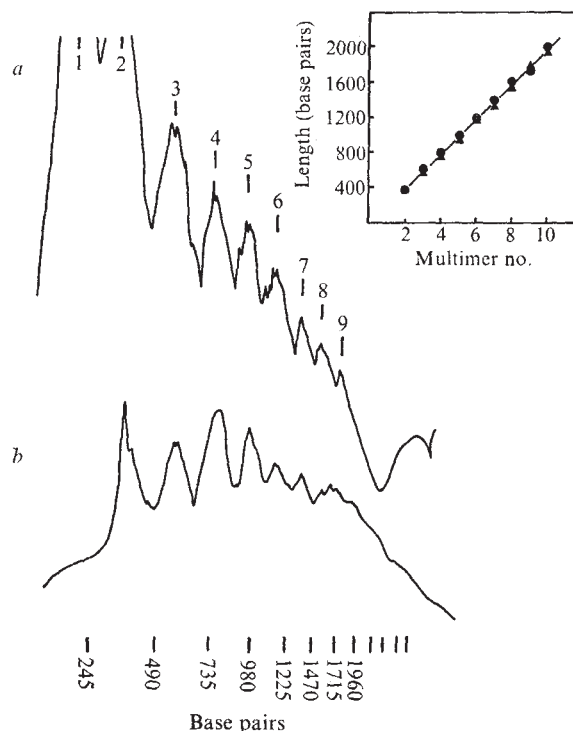


Fig. 1 Hybridisation of ^{32}P -cDNA transcribed from mouse liver poly A-containing RNA to nucleosomal DNA. *a*, Densitometer scan of a photograph of the DNA nucleosome repeat pattern resolved in an agarose gel. The peaks represent amounts of DNA shown by ethidium staining. Nucleosome multimer numbers are given. *b*, Densitometer scan of ^{32}P -cDNA hybridisation autoradiogram. Mouse liver nuclei were prepared¹⁹ and digested with micrococcal nuclease (200 units ml^{-1}) at 37°C for 5 min at a nuclear DNA concentration of 2 mg ml^{-1} . DNA from this digest (17 μg) was electrophoresed at 2 V cm^{-1} for 16 h in a 1.7% agarose gel⁵. Mouse satellite DNA, isolated by CsSO_4 -silver buoyant density centrifugation, was digested with *EcoRII* (0.17 units) in 50 mM Tris (pH 7.5), 5 mM MgCl_2 for 30–60 min at a DNA concentration of 150 $\mu\text{g ml}^{-1}$. 1- μg aliquots of this digested satellite DNA were subjected to electrophoresis in slots adjacent to those containing DNA from the micrococcal nuclease digestion. Positions and sizes of the restriction fragments¹⁸ of this DNA are shown at the bottom. After staining with ethidium bromide, the gel was photographed with a red filter under ultra-violet light. The DNA in the gel was then denatured *in situ*, transferred to nitrocellulose¹⁵, and processed for hybridisation²⁰. ^{32}P -cDNA to mouse liver poly A-containing RNA was synthesised²¹ with AMV reverse transcriptase yielding a specific activity of 8×10^6 c.p.m. μg^{-1} . 1 μg of ^{32}P -cDNA and 20 μg of carrier tRNA were dissolved in Denhardt solution containing $6 \times \text{SSC}$ and 0.5% SDS²⁰, and denatured by heating for 5 min at 95°C . Hybridisation to DNA immobilised on nitrocellulose was carried out at 68°C for 24 h. Little or no hybridisation of cDNA to the monomer nucleosome band was observed; this could be due to loss of monomer DNA from the gel during washing steps before transfer to nitrocellulose^{15,20}. Inset, plot of multimer band number against DNA fragment lengths for nucleosome multimers (Δ) and for ^{32}P -cDNA hybridisation ($\bullet$). Fragment lengths were determined by calibration with satellite DNA restriction fragments¹⁶. The length of DNA in the nucleosomes of mouse liver is 195 ± 5 base pairs; similarly, the ^{32}P -cDNA hybridises to DNA with a repeat length of 197 ± 7 base pairs.

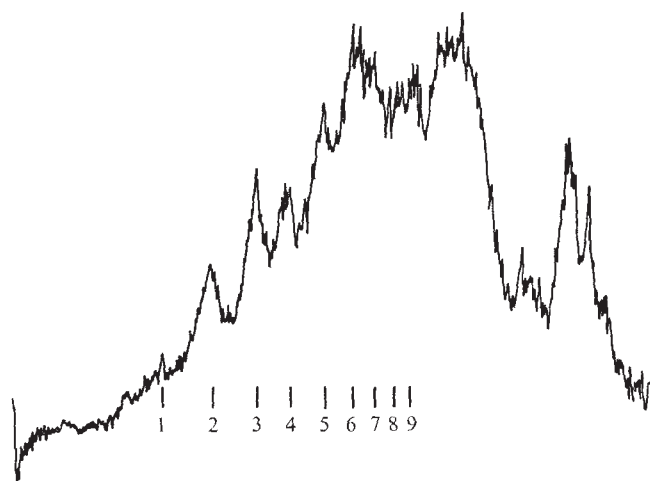


Fig. 2 Hybridisation of ^{125}I -labelled mouse ribosomal RNA to nucleosomal DNA. DNA, isolated from a partial micrococcal nuclease digest of mouse liver nuclei, was electrophoresed in Agarose and transferred to nitrocellulose as described in Fig. 1. Ribosomal RNA was labelled by iodination²², yielding a specific activity of 6×10^6 c.p.m. μg^{-1} . $1 \mu\text{g}$ of this RNA was hybridised to nitrocellulose-immobilised nucleosomal DNA²⁰. The densitometer scan shown was obtained from the hybridisation autoradiogram. The numbers correspond to the position of nucleosome multimer bands in the original Agarose gel as seen by ethidium staining. The nucleosome repeat length of hybridised rDNA is 195 ± 5 base pairs.

We have devised the following protocol to determine the nucleosome repeat length of any DNA sequence for which a radioactive probe is available. First, DNA from a partial micrococcal nuclease digest of nuclei is electrophoresed in an Agarose gel, thereby revealing the typical nucleosome band pattern. This DNA is denatured *in situ* and then transferred to nitrocellulose by the blotting technique of Southern¹⁵. Next, a radioactive probe is applied in conditions which allow hybridisation to complementary sequences immobilised on the nitrocellulose sheet. The nitrocellulose is washed free of nonhybridised probe and an autoradiogram made. A densitometer tracing of this autoradiogram graphically represents the position and quantity of hybridised nucleic acid.

We have used a ^{32}P -labelled cDNA of mouse liver poly A-containing nuclear RNA as a probe for transcribed DNA sequences. The densitometer scans in Fig. 1 show both the nucleosome pattern of total DNA from a micrococcal nuclease digest of liver nuclei and an autoradiogram of the ^{32}P -cDNA hybridisation to the DNA of this digest. The positions of peaks of hybridisation are coincident with the peaks of nucleosomal DNA in the Agarose gel. This demonstrates that transcribed sequences are indeed represented in nucleosomal DNA³⁻⁸ and that the average repeat length of transcribed DNA is the same as the average repeat length of mouse liver chromatin DNA (195 ± 5 base pairs). It should be noted that in these limited nuclease digestion conditions (Fig. 1), it is highly unlikely that actively transcribed DNA sequences are not represented in the nucleosome band pattern. Only about 3% of nuclear DNA is rendered acid soluble in our conditions.

Poly A-containing RNA of liver represents a diverse population of nucleotide sequences, some of which are presumably the products of continuous transcription, while others are the products of less frequent or even rare transcriptional events. Thus, it is unlikely that the hybridisation pattern depicted in Fig. 1 comes only from the annealing of ^{32}P -cDNA to DNA sequences which are actually engaged in transcription at the time the cells were disrupted. Rather, it is more likely that the hybridisation represents ^{32}P -cDNA annealing to sequences which are transcribed at some point in the cells' history.

We have also examined the repeat length of ribosomal

RNA-coding sequences (rDNA) by hybridisation of ^{125}I -labelled rRNA to a micrococcal nuclease digest of liver nuclei (Fig. 2). Here again, the distribution of hybridisation parallels that of nucleosomal DNA. Several authors have reported that rDNA sequences are contained in nucleosomes⁶⁻⁸. Our data confirm these reports and demonstrate that the nucleosome repeat length of rDNA is 195 ± 5 base pairs in mouse liver. As rDNA sequences are repetitive, it is possible that some copies of rDNA are not active in transcription in the liver. Thus, the annealing of rRNA depicted in Fig. 2 could be due to hybridisation in inactive as well as to transcriptionally active rDNA sequences.

We have used mouse satellite DNA as a probe for non-transcribed sequences. No RNA transcripts of satellite DNA have been detected in mouse liver, kidney or spleen¹⁶; *in situ* hybridisation shows the satellite DNA to be concentrated in centromeric heterochromatin¹⁷. In agreement with these results, we find that ^{32}P -labelled cDNA to poly A-containing nuclear RNA does not hybridise to satellite DNA (J.M.G. and D.A.M., unpublished). The densitometer scans in Fig. 3 show the hybridisation of ^{32}P -labelled mouse satellite DNA to liver nucleosomal DNA and to an *Eco*RII digest of satellite DNA. The distribution of hybridised ^{32}P -satellite DNA parallels the distribution of nucleosomal DNA in the original Agarose gel. This demonstrates that the average nucleosome repeat of mouse satellite chromatin is 195 ± 5 base pairs. Thus, both transcribed and nontranscribed DNA sequences of mouse liver have the same nucleosome repeat length.

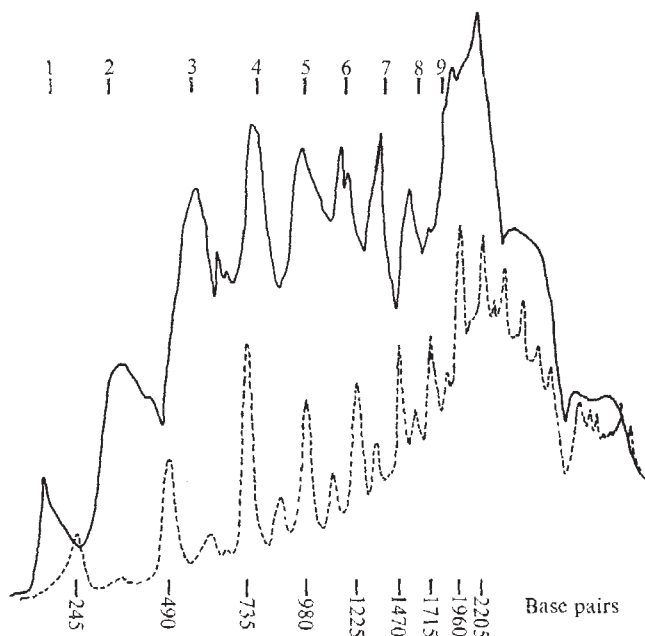


Fig. 3 Hybridisation of ^{32}P -labelled satellite DNA to nucleosomal DNA. Solid line, densitometer scan of autoradiogram of satellite DNA hybridisation to DNA from a micrococcal nuclease digest of mouse liver nuclei. Dotted line, densitometer scan of autoradiogram of satellite DNA hybridisation to *Eco*RII-digested satellite DNA immobilised on nitrocellulose. The positions of the nucleosome multimers in the original Agarose gel are indicated at the top. The sizes and positions of the major satellite DNA restriction fragments are given at the bottom. Note that the positions of satellite DNA hybridisation to nucleosomal DNA are coincident with the nucleosomal DNA bands (195 ± 5 base pairs) and not at positions corresponding to the satellite DNA repeat unit (245 base pairs). Mouse satellite DNA was labelled by nick translation²³ with *Escherichia coli* DNA polymerase I (25 units; Boehringer) in the presence of $30 \mu\text{Ci}$ of ^{32}P -dATP (250 Ci mmol^{-1} ; Amersham) and unlabelled dCTP, dTTP and dGTP at a concentration of $6 \mu\text{M}$ each, $0.6 \mu\text{g}$ of this DNA (6.6×10^6 c.p.m. μg^{-1}) was used for hybridisation (as described in Fig. 1). The specificity of hybridisation was confirmed by annealing in more stringent conditions ($2 \times \text{SSC}$ at 68°C); results similar to those shown ($6 \times \text{SSC}$ at 68°C) were obtained.

Notably, the nucleosome repeat length of mouse satellite DNA is not the same as the DNA sequence repeat. Mouse satellite DNA has a sequence repeat every 245 base pairs, as indicated by the periodicity of restriction nuclease sites (Fig. 3 and ref. 18). We have shown above that the nucleosome repeat is 195 base pairs (Fig. 3). Thus, there is no simple relationship between the nucleotide sequence repeat in mouse satellite DNA and the organisation of these sequences in nucleosomes. This finding is in contrast to the results of Musich *et al.*¹⁰ who report that component α DNA of African green monkey has identical nucleosome and DNA sequence repeats (176 base pairs for both). They suggest that a precise register of chromatin proteins and DNA might exist for component α DNA. Our results and those of Horz *et al.*¹² show that the similarity of nucleosome and sequence repeat lengths is not universal for highly repetitive DNAs.

In conclusion, we have shown that both transcribed and nontranscribed DNA sequences are organised in nucleosomes with the same average repeat lengths, and that the nucleosome repeat for mouse satellite DNA, 195 base pairs, is not the same as its DNA sequence repeat, 245 base pairs¹².

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Table 1 α/β mRNA ratios determined by hybridisation of cDNA _{α} and cDNA _{β} to total cytoplasmic RNA

Source of total cytoplasmic RNA	α/β mRNA ratio
Normal European control (average of 3 samples)	1.41
Iron deficiency control (average of 3 samples)	1.32
HbH disease	0.45
α thal 1 heterozygote	0.69
α thal 2 heterozygote	0.97
Normal Caucasian control (taken and transported with Melanesian samples)	1.41
HbJ Tongariki homozygote	0.70
HbJ Tongariki heterozygote	0.78
HbA-only Melanesian control	0.62

cDNA _{α} and cDNA _{β} were hybridised as described in Fig. 1. The ratio of mRNA _{α} /mRNA _{β} is equal to the RNA/cDNA ratio at which 50% of the cDNA _{α} is hybridised, divided by the ratio at which 50% of the cDNA _{β} is hybridised after each cDNA concentration has been corrected for the maximum amount of hybridisable cDNA.

that some, if not all, Melanesians have only one α chain locus. However, as recent studies have shown that two of the common forms of α thalassaemia are due to a gene deletion⁶⁻⁸, the disease must be quite rigorously excluded before the number of α gene loci can be deduced solely on the basis of genetic evidence. We have studied again the family described by Beaven *et al.*, and other unrelated individuals, all of whom live on Karkar Island, off the northern coast of Papua New Guinea. By determination of α/β mRNA ratios in these individuals we have obtained evidence which strongly suggests that they are in fact homozygous for an α thalassaemia gene.

Blood samples (10 ml in heparin) were transported to Oxford within 72 h of collection, and immediately on receipt total RNA was isolated from the bulk of the washed cells by extraction with phenol-chloroform. Starch-gel electrophoresis of haemolysates showed no trace of HbA in the HbJ homozygote, and 40-60% HbJ in the heterozygotes. The blood films of the seven Karkar subjects showed mild storage artefacts. Red cell indices were as follows: MCV 55-65 fl; MCH 18.1-22.8 pg; MCHC 31.7-34.5 g per 100 ml. HbF and HbA₂ values were normal. In contrast, a Caucasian control sample, taken on Karkar and transported with the test samples, had normal indices (MCV 86 fl; MCH 31.9 pg; MCHC 36.5 g per 100 ml) but the red cells also showed mild storage artefacts, and it thus seems unlikely that the low indices in the Melanesian samples were caused by storage.

The red cell indices of the Melanesian samples are typical of 'severe' α thalassaemia trait (α thal 1), but can also be seen in iron deficiency anaemia. As it was not possible to determine plasma iron levels we cannot rule out iron deficiency as an explanation. However, as we show below, α/β mRNA ratios in reticulocytes from patients with iron deficiency fall within the normal range, whereas in carriers for α thalassaemia they are significantly reduced.

In addition to the samples from Karkar we studied as controls three normal individuals, three patients with iron deficiency anaemia, the parents of a Cypriot patient with typical HbH disease, and a patient with HbH disease.

α/β mRNA ratios were measured by hybridisation of total RNA to purified complementary DNA (cDNA) probes specific for α and β mRNA sequences⁷. The normal controls had α/β ratios which ranged from 1.26-1.45 (Table 1, Fig. 1a). The three iron deficiency anaemia samples also fell within this range (Table 1). Thus, although iron deficiency may affect the relative rates of α and β chain synthesis by limiting the availability of haem, which may alter relative rates of α and β chain initiation⁹, it does not influence the proportions of α and β mRNA in affected cells.

Haematological studies on the parents of a patient with HbH disease showed α thal 1 trait in the mother, whereas

Haemoglobin J Tongariki is associated with α thalassaemia

GENETIC evidence suggests that the haemoglobin (Hb) α chain genes are duplicated in at least some human populations, as a number of individuals are known to have two different α chain variants in addition to HbA (ref. 1). Furthermore the presence of two α chain genes per haploid genome has been demonstrated in a few cases using direct measurement by DNA hybridisation^{2,3}. However, this may not be true of all racial groups, particularly the Melanesian populations in which HbJ Tongariki is found. Abramson *et al.*⁴ and Beaven *et al.*⁵ described five individuals who were apparently homozygous for this α chain variant, none of whom had any detectable HbA. α Thalassaemia was discounted on haematological grounds, and it was concluded

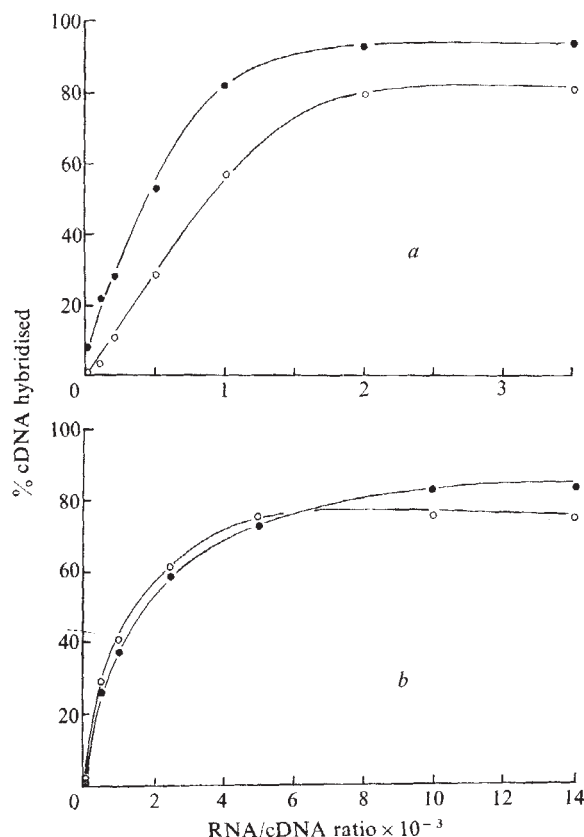


Fig. 1 Hybridisation of cDNA_α and cDNA_β to total cytoplasmic RNA isolated from normal reticulocytes (a) and HbJ Tongariki homozygote peripheral blood (b). cDNA_α and cDNA_β were prepared by the hybridisation of ³H-cDNA_{αβ} (prepared as described previously¹⁴ using an RNA-dependent DNA polymerase purified from avian myeloblastosis virus) to β⁰/δ⁰β⁰ thal mRNA which lacks mRNA_β sequences¹⁵, and separating the hybridised component (cDNA_α enriched) from the non-hybridised component (cDNA_β enriched) by hydroxylapatite fractionation at 60 °C (ref. 2). The cDNA_α probe hybridised to mRNA_{αβ} to 90% completion (a) and contained approximately 100% cDNA_α as determined by hybridisation to mRNA lacking α-globin sequences (unpublished). The cDNA_β probe hybridised to mRNA_{αβ} to 80% completion (a) and contained 12% cDNA_α as determined by hybridisation to mRNA lacking β-globin sequences (unpublished). Total cytoplasmic RNA from peripheral blood was prepared by phenol-chloroform extraction. 100 pg cDNA_α (●) or cDNA_β (○) were mixed with increasing amounts of RNA in 2 μl hybridisation buffer, incubated at 43 °C for 100 h, and the amount of cDNA hybridised determined with S₁ nuclease as described previously¹⁴.

the father had an essentially normal blood picture, compatible with him being a carrier of the 'silent' α thal 2 gene¹⁰. The rates of α and β chain synthesis were measured as described previously¹¹. In the mother the α/β ratio was 0.59, whereas the father's ratio was 0.84. Both these values fall within the ranges for α thal 1 and α thal 2 heterozygotes determined previously in this laboratory¹². The mRNA ratios showed a similar pattern. HbH disease RNA had an α/β ratio of 0.45. In the α thal 1 heterozygote it was 0.69, whereas in the α thal 2 heterozygote it was 0.97, values which are reflected by the respective α/β chain synthesis ratios.

In the Oriental and Mediterranean populations in which α thalassaemia is common, normal individuals have two α genes per haploid set¹. In α thal 1 both α genes are deleted from the chromosome^{6,7}, whereas in α thal 2 one of the two genes is deleted⁸. Thus carriers for α thal 1 and α thal 2 have, respectively, only two or three α genes instead of the usual four, and in HbH disease, in which α thal 1 and α thal 2 interact, there is only one α gene. These α gene deficiencies are reflected in the measured α/β mRNA ratios.

Hybridisation of homozygous HbJ Tongariki RNA to purified cDNA_α and cDNA_β probes is shown in Fig. 1b. Clearly the rate of hybridisation of cDNA_α is slower than for cDNA_β, when compared with hybridisation of RNA from a normal individual to the same cDNA probes (Fig. 1a). The α/β mRNA ratio, calculated according to Fig. 1, was 0.70. Similar experiments were carried out using RNA from a HbJ Tongariki heterozygote and from a 'normal' Karkar islander (that is, one possessing only HbA). In each case there was marked deficiency of α mRNA relative to β mRNA, the actual α/β mRNA ratios being similar to those found in the HbJ Tongariki homozygote and in the Cypriot α thal 1 control (Table 1).

These results reveal a marked reduction in α mRNA levels in the Karkar samples, similar to that seen in individuals heterozygous for α thal 1, in whom only two out of the normal four α genes are present. The haematological findings are also compatible with the presence of α thalassaemia in these individuals. The data imply, however, that they must be homozygous for whatever α thalassaemia gene they carry, as the α/β mRNA ratios and haematological findings are the same for all individuals, and independent of the presence or absence of the gene for HbJ Tongariki. The most obvious explanation is that some, perhaps many, 'normal' individuals in this population are homozygous for an α thal 2 gene and thus have only one functional α gene on each chromosome, and that the α^J Tongariki genotype arose by a mutation at this single α chain locus.

It is perhaps surprising that a homozygous α thal 2 genotype should be so common in this Melanesian population. Possibly its penetration is complete, although too few individuals have yet been studied to be able to rule out the occurrence of any normal two-α-gene genotypes in Melanesians. Its high incidence may be connected with the existence of endemic malaria on many of these islands¹³, which might favour the rapid selection of thalassaemia genes, particularly among small isolated racial groups in which inbreeding is presumably common. Further studies are obviously required to clarify some of the points raised here and we are currently collecting more samples on Karkar for this purpose.

We thank Dr Robin Carrell for his help, A. McGregor for collecting the samples on Karkar, and the MRC for financial support. We are indebted to Dr J. Beard, Life Sciences Research Laboratories, St Petersburg, Florida, for the gift of reverse transcriptase.

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reviews

Cancer research in the US

Robert Q. Marston

Cancer Crusade: The Story of the National Cancer Act of 1971. By R. A. Rettig. Pp. 382. (Princeton University Press: Princeton, New Jersey, 1977.) \$15; £10.10.

A PROPER review of the impact of the US National Cancer Act of 1971 would require the passage of more time as well as the pen of one who was not an active player in the drama. Yet seven years is not as short a time as it once was in science, and most people mentioned in this book can find solace in the even-handed way personalities are handled. Indeed I, for one, would change no word of mine quoted. Benno Schmidt, Chairman of the President's Cancer Panel, writes to me regularly defending positions previously taken; Senator Gaylord Nelson received only praise for his single negative vote in the US Senate; and Representative Paul Rogers and Senator Edward Kennedy have retained the prominence in health matters which they had during the great cancer debate.

Mr Rettig's *Cancer Crusade* will appeal most to those interested in cancer, in biomedical research, in science policy, and in American politics. Although the issues are deadly serious and the stakes high, there is little evil and as many heroes as one's own prejudices allow. The issues are drawn clearly and are of universal and historical interest. The adversaries were powerful and determined to have their way.

The story starts, in Mr Rettig's words, when "In late 1970, a relatively obscure group called the National Panel of Consultants on the Conquest of Cancer presented a report entitled 'National Program for the Conquest of Cancer' . . . One year later on December 23, 1971, President Richard M. Nixon signed into law the National Cancer Act of 1971".

Between these two events debate centred on two of the three main recommendations of the Panel of Consultants. First, the Panel recommended the establishment of a new government agency—the National Cancer Authority—the mission of which would be defined by statute as "the conquest of cancer at the earliest possible time". The Authority would absorb all functions of the National Cancer Institute

(NCI) and would be completely independent of the National Institutes of Health (NIH). The second recommendation was that "a comprehensive national plan for the conquest of cancer", reminiscent of the space plan, be developed. Finally, the panel recommended a greatly expanded budget for cancer research. Although a budget of $\$800 \times 10^6$ to $\$1 \times 10^9$ per year was proposed, this led to little debate.

The setting of the debate is accurately described by Mr Rettig to include the post-Vietnam austerity period of slackened growth in the support of biomedical research, a call for more relevant research to produce practical results faster, and a degree of disillusionment with science generally. Indeed, a major goal of the Panel of Consultants was to regain momentum for biomedical research support, but especially for cancer research.

Opposing the recommendations of the Panel to move cancer research out of the NIH and to carry out anything even remotely similar to a 'moon shot', were the NIH and the vast majority of the biomedical scientists, many of them working in the universities of the United States.

The book states that immediately after the release of the report, the Director of the NIH responded to a question about the separation of the NCI from the NIH, "I'm against it", and four days later he wrote:

"The separation of the substantive scientific programs of cancer research from other biomedical research endeavors would be counterproductive to progress in cancer and other research. At the present time, various Institutes and Divisions of the NIH are engaged in and support research work that is highly relevant to the areas of special promise listed by the Panel. The virologists, immunologists, cell biologists, epidemiologists, pharmacologists, and others involved in such studies are focussed on fundamental life processes, which are undoubtedly vital to the understanding of cancer even though they may be addressing their research problems through different motivations."

The fact that the NIH is organised by disease categories—cancer, heart, arthritis, infectious diseases, and so on—and can easily be seen to be a mission-oriented federal health agency in no way beclouds its international recognition as a first-class scientific

institution. Federal scientists at NIH headquarters in Bethesda, Maryland, and their thousands of counterparts supported by NIH grants at universities and research institutions throughout the nation believe that truly significant progress against serious diseases will come primarily from investigator-initiated research—good research conceived of and carried out by excellent scientists—rather than from centrally planned 'targeted research'.

Parenthetically, this view is shared widely throughout the world by medical research councils and biomedical scientists. Yet all have mused, along with those at NIH, about possibly better ways to organise and manage research. Thus the cancer debate in the US was followed widely and discussed frequently with leaders such as Sir John Gray, then Secretary of the British Medical Research Council.

One of the strengths of the book is the treatment of the role of the group Mr Rettig calls "the Benevolent Plotters". Mrs Mary Lasker, the leader, is credited by Mr Rettig as contributing more to the Panel of Consultants, than any other individual. I first heard of Mrs Lasker in 1948 as we walked across University Parks, Oxford, from Sir Howard Florey. He mentioned she had supported research in the Sir William Dunn School following the development of penicillin there.

Mr Rettig explores at some length how "a small but powerful elite group composed of private citizens mobilised sufficient political resources to secure passage of legislation opposed by the NIH and by most of the biomedical scientific community". He concludes that the skill of Mrs Lasker, Mr Benno Schmidt (Chairman of the Panel) and others resulted in a classic example of the effective moulding of public policy. Their efforts built, however, on the deep-seated fear of cancer, coupled with the somewhat naive but widely held view that science can make things come out as we would like them to be.

He offers the dramatic example of the effect of Ann Landers' column on the American public and through them on the Congress of the United States. Ann Landers normally limits her daily newspaper syndicated column to advice for family problems. Listen to the de-

scription of this particular column as detailed by Mr Rettig:

"A number of stories related to cancer appeared in March and April, but the most significant publicity occurred in mid-April, when Ann Landers devoted an entire column to urging support for S34. 'Dear Readers,' she began, 'if you are looking for a laugh today, you had better skip Ann Landers'. But, 'if you want to be part of an effort that might save millions of lives—maybe your own—please stay with me'. She asked, 'Who among us has not lost a loved one to cancer?' If enough citizens let their senators know that they want the bill—S34—passed, it will pass'."

That single column resulted in more than 1,000,000 letters and telegrams to Senators and Congressmen.

A highlight of the entire drama—especially for democratic societies—was the impact of the Senate vote on House of Representatives action. Had the Senate vote been 80-0 rather than 79-1, it is probable the story would have been significantly different. I remember clearly the night Senator Gaylord Nelson decided that standing alone would be good science and good political statesmanship.

Senator Nelson's decision made Representative Rogers and his committee's action possible. Mr Rettig describes with great accuracy the role of the various scientific and academic associations, led by the Association of American Medical Colleges, in providing the congressman with information and testimony to justify significant modification of the Senate Bill.

Although the National Cancer Act of 1971 was a compromise of many actions by many parties over many months, Representative Rogers of Florida emerged as the advocate for the scientific community. He reported the Act as "basically the bill which the House approved". "Mr Speaker", he said, "this report represents a substantial victory for the House of Representatives, the biomedical community and the American people".

In the Act finally passed, the NCI remained a part of the NIH, with the Director of the NCI still reporting to the Director of the NIH for most purposes. However, among other less important changes, the budget of the NCI now went unchanged from the Director of the NCI to the President. A special Presidential panel of three persons was established to oversee the NCI, and the Act provided that both the Director of the NIH and the Director of the NCI be appointed by the President.

Perhaps the weakest part of this book is the attempt by the author to analyse the current and future issues raised by the Act. Yet this must be the case, because we really don't know even today whether the Cancer Crusade

was a good idea or bad—and Mr Rettig can't tell us. My main criticism of the book is a criticism of the debate itself. It was too far removed from the substance of science for my liking. Viral oncology was fading as a key factor in the fight against human disease, even as inbred animal models with virus-induced cancers were trumpeted as possible answers to our hopes. Environmental pollutants were emerging as troublesome factors with high price tags in costs and personal liberty, even as suggestions were made that the price was dollars, not self denial or tradeoffs with other major social desirables.

Those who saw the Cancer Crusade as outstripping the science base and thus constituting overpromise of an unachievable goal at unreasonable costs and at the expense of other desirables may well be right. However, science is always unpredictable.

On the crucial debate about the separate nature of the NCI the issue seems resolved, at least in the mind of the current Director of the NCI, Dr Arthur Upton, who said recently:

"I report to NIH Director Don Fredrickson, Assistant Secretary Richmond, and Secretary Califano just as any other Institute Director. Regarding the so-called budget bypass, it is a great advantage for NCI to be able to submit its budget directly to the White House and the Office of Management and Budget but within this administration cancer program matters have been delegated directly to Secretary Califano. So now the Department of Health, Education and Welfare is much more in the picture."

On the other hand, the budget of the NCI has increased greatly, reversing a more stable trend of seven years ago. It will be surprising indeed if this in-

creased effort does not increase our understanding of cancer and other life processes.

The Cancer Crusade was, and is, an event of major interest. A fair question would be how does it compare with other issues at the NIH in these years? Symbolically, it was not in the class as the several Nobel Prizes and other high awards in the intramural programs and in the nation as American biomedical science came of age. From a substantive standpoint the whole DNA/RNA story has raised more new issues and made far more demands on the time of the Director. From the practical standpoint, the depressed mortality statistics possibly related to changes in life style—smoking, diet, exercise—are surprisingly early in coming (if true). Training grants and full funding of non-competing grants were great, although less dramatic issues. In short, the great cancer debate was one of several important issues of the late 1960s and early 1970s at the NIH.

The book ends with the subchapter title, "Transition to a New Era?" However, I fear a new era in which the question is science or no science, not the almost joyous debate of how best to find the truth. I fear adversaries who are not noble and competent, but who are mean-minded and self-seeking. I fear a world in which the outcome of experiments are manipulated, not where the outcome is debated. I fear a world in which a crusade is never launched even if, as often is the case, it may be imperfectly conceived. □

Robert Q. Marston was Director of the NIH from 1968-73, and is now President of the University of Florida.

Cosmical magnetic fields

Magnetic Field Generation in Electrically-Conducting Fluids. By H. K. Moffatt. Pp. 343. (Cambridge University Press: Cambridge and London, 1978.) £15.50.

"How could a rotating body such as the Sun become a magnet?" was the title of Sir Joseph Larmor's celebrated talk at the 1919 meeting of the British Association for the Advancement of Science. He proposed in that lecture that the magnetic field of the Sun was due to ordinary electric currents in the solar interior and that the electromotive forces required to maintain these electric currents against ohmic dissipation were due to inductive interaction between fluid motions in the solar interior and the magnetic field

itself. Geophysicists now accept that the main geomagnetic field is produced in a similar way, by a 'self-exciting dynamo' mechanism operating in the molten core of the Earth, and the dynamo process has also been invoked to explain the more recently discovered magnetic fields of Jupiter and other planets. Unlike the dynamo process, other proposed mechanisms for generating the magnetic fields of these astronomical bodies are much too feeble; only the dynamo theory holds out any promise of final success.

The main difficulty with dynamo theory was exposed by Cowling when, in 1933, he demonstrated that the particular configuration of fluid motion and magnetic field invoked by Larmor was one of a more general class that are incapable of producing dynamo action. The subsequent pioneering work on the dynamo theory of the Earth's magnetism, by Bullard, Elsasser,

Parker and others, had therefore to be carried out without the support and comfort of an 'existence theorem' demonstrating that flow and field configurations of a 'non-Larmor' type in a simply connected electrically conducting body of fluid could produce dynamo action. Fortunately for the development of the subject, Backus and Herzenberg were able in 1958 to provide two independent proofs of such a theorem, and research towards the elucidation of the details of the dynamo mechanism has grown rapidly since that time.

The method of attack is almost entirely mathematical, because of the practical difficulty of attaining sufficiently high values of the so-called 'magnetic Reynolds number' R with available laboratory fluids. (R is defined as the product of four quantities: the electrical conductivity, magnetic permeability, typical speed of fluid flow and typical length scale of the system; it is thus quite high even for poor conductors on the length scale of astronomical systems, and this is why the study of 'magnetohydrodynamics' first emerged from astrophysical investigations.) Several excellent reviews of dynamo theory have appeared in the specialist literature during the past few years, but Professor Moffatt has provided the first monograph setting out "the general mathematical theory of magnetic field generation by inductive fluid motion, with particular reference to the two most accessible examples of cosmic bodies (Sun and Earth) exhibiting magnetic fields", with the objective of placing a wide range of work within a common framework and clarifying areas of overlap as well as areas of conflict in this technically highly complex subject.

A dominant idea which recurs throughout this book, which is based on a graduate lecture course given over a number of years at Cambridge, is the lack of reflexional symmetry of fluid flows capable of producing dynamo action; in short, typical fluid eddies in 'non-Larmor'-type configurations have a helical structure. A measure of this lack of reflexional symmetry is the pseudo-scalar quantity 'helicity', defined as the scalar product of the flow velocity vector and the local 'spin' vector (vorticity) of the fluid. In the author's words, "In a sense, this is a book about helicity; the invariance and topological interpretation of (helicity) are discussed at an early stage (chapter 2) and the central importance of helicity in the dynamo context is emphasised in chapters 7 and 8. Helicity is also the main theme of chapter 10 (on helical wave motions) and of chapter 11, in which its influence on turbulent flows with and without magnetic fields is discussed".

As far as the rest of the book is concerned, chapter 3 deals with the convection, distribution and diffusion of magnetic fields, and chapters 4 and 5 are accounts of the magnetic fields of the Earth and Sun, with a few remarks about the fields of other planets. Chapters 6 and 9 deal respectively with laminar and turbulent "kinematic dynamos", in which the field of fluid flow is specified *a priori* in the calculation of magnetic effects from the equations of electrodynamics. It is with this part of the subject that the most striking progress has been made during the past decade by workers in several countries, including the Soviet Union and the German Democratic Republic. The final chapter indicates what little work has been done on the much more difficult problems arising from the investigation of 'magnetohydrodynamic dynamos' which seeks simultaneous solutions of the equations of hydrodynamics and electrodynamics.

Progress report on perception

The Perceptual World. By Kai von Fieandt and I. K. Moustgaard. Pp. 680. (Academic: New York, London and San Francisco, 1978.) \$62.50; £32.

DRAW a rough sketch of what could be called a map of knowledge. Physics and astronomy would share common frontiers with chemistry and geology which in turn have borders with molecular biology and physiology. There are many disciplines each of which has its own set of concepts and language. For some subjects, however, it is not always clear where the borders are; for example, modern linguistics merges imperceptibly into terrains where philosophy, mathematics and logic have traditionally held sway. Nowhere is this problem more acute than for psychology. Suffice to say its subject matter is vast; its methods and procedures are parasitic upon many other disciplines, but at the same time it is not always clear what it is that constitutes a problem for the psychologist. But psychology does have one concern where a clear picture of its problems are emerging and where there are now concepts and a language appropriate to it. This area of psychology is what is generally referred to as perception. Perceptual psychology has two concerns: first, how events in the external world become stored as information in the brain, and second, how this stored information becomes knowledge of the world. Psychologists have made great strides with the first problem but the second still awaits clarification from philosophers and workers in artificial intelligence.

This book is certainly not aimed at the mathematically faint-hearted but, in the tradition of the Cambridge University Press monographs on Mathematics and Applied Mathematics edited by Professors G. K. Batchelor and J. W. Miles, the subject is introduced in an interesting way, and the mathematical equations and derivations are well supported by physical ideas and explanations. The book contains a few misprints and errors (for example Herzenberg's famous paper is inadvertently omitted from the list of references) and can be recommended to anyone interested in the nature of cosmical magnetic fields, which Einstein evidently regarded as presenting one of the most important unsolved problems in physics.

R. Hide

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The Perceptual World, written by psychologists, is a progress report of work in perception. This work is backed up by the insights and methods from colleagues in many other disciplines. John Mollon's two chapters indicate the scope of the enterprise. He discusses neural coding and analysis, and in doing so, annexes to the service of perceptual psychology large areas of what was previously thought to belong exclusively to the province of neurophysiology. But he does this to good effect; he shows, for example, that at the neural level an account can be given of how external events are detected, coded and stored in the brain. This ecumenical spirit pervades the book, and so we find that the chapter on psychophysics draws on signal detection theory, which was originally developed by communication engineers to describe information flow in noisy systems.

Many books on perception deal all too exclusively with the input side—with the collection coding and storage of information—to the neglect of the output which issues as behaviour and experience. *The Perceptual World* avoids this fault: there are chapters dealing with object perception and the perception of self and these chapters therefore have something to say about knowledge of the world. This final section of the book discusses the experience of perception, and how this interacts with the perceiver's personality, life history and culture. The chapter on pictorial art is a *tour de force*, and manages to be original and interesting and to avoid the woolly inanities which so often pass for discussion on this topic.

William Barnes-Gutteridge

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Phosphorus chemistry and technology

Phosphorus: An Outline of Its Chemistry, Biochemistry and Technology. By D. E. C. Corbridge. Pp. 455. (North-Holland: Amsterdam, New York and Oxford, 1977.) \$59.60; Dfl.146.

THE chemistry of phosphorus is second only to that of carbon in its richness and diversity. Writing a single-volume text is thus a hazardous as well as an ambitious undertaking. The inevitable omissions and compressions can scarcely fail to disappoint specialists in the field. On the other hand, most readers prefer to use a single volume as the way in to a new subject, in the hope that they will find the answers to simple questions, and references to the secondary literature.

This book represents a brave attempt to cover "all aspects of phosphorus chemistry: organic, inorganic, biochemical, physical, environmental and technical". The level is intended to be basic and introductory; defined here as accessible to readers with at least one year of university chemistry. It is thus in direct competition with Emsley and Hall (*The Chemistry of Phosphorus*, reviewed by R. A. Shaw in *Nature*, **264**, 302; 1976).

Happily the two approaches are largely complementary. Dr Corbridge gives us a traditional treatment, packed with factual information, which is particularly strong on technological, inorganic and structural chemistry (the book has clearly developed out of the same author's *Structural Chemistry of Phosphorus*), whereas Emsley and Hall are particularly stimulating on organic and environmental aspects. How to deal with the biochemistry of phosphorus is a traditionally difficult problem, which neither book solves very satisfactorily. Dr Corbridge provides a long chapter, which illustrates very clearly the strengths and drawbacks of his approach. Everything of importance gets a mention: terms are meticulously defined even where reference to standard biochemical texts might seem more appropriate ("Enzymes are a special type of catalyst which are part protein . . ."); and compounds are evidently real things, not just formulae on paper ("Absolute glycerophosphoric acid . . . is a colourless, odourless, syrupy liquid . . .").

On the other hand, interpretation, and especially speculation, is practically absent: reaction mechanisms for example, are fairly rudimentary. This has helped to keep down the size of the book, at the expense of much of the sense that phosphorus chemistry can be intellectually exciting. What does come across very clearly, however, is the author's fascin-

ation with the subject. The history of phosphorus is nicely sketched in, and the ten-page description of the properties of the element beautifully done. The reader who wants to know what safety matches are made of will not be disappointed, and as a bonus will also find out about toy pistol caps.

A workman-like book, then, with solid virtues; but not a beautiful book. The cover, in four shades of purple with green spots, will not be to everyone's taste. And reproduced typescript is always unattractive; though at least the number of words per page compares not unfavourably with similar printed books (partly a result of using smaller type for specialised passages). Errors, too, are more plentiful

than one would like; from harmless, even amusing, typing errors (lethicin; lithiosphere; and even, enzymes that can be used "in vitrio") to the more serious problem of incomplete structural formulae in a number of places.

A well-stocked chemistry library will want to have this book, which works well on its own terms as a basic introduction to the subject. As such there is also a case for it to be in more general reference libraries which have nothing up to date in the area.

A. J. Kirby

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Interactions between molecules

Intermolecular Interactions: From Diatomics to Biopolymers. Edited by Bernard Pullman. Pp. 447. (Wiley: London, New York and San Francisco, 1978.) £19.50.

MOLECULAR structure is, from a theoretical point of view, essentially a solved problem. The reactivity of molecules on the other hand remains beyond the scope of theoretical calculation. The interactions between molecules are a half-way house and Bernard Pullman, with his customary nose for a good problem, has chosen this topic for a second volume of his series on perspectives in quantum chemistry and biochemistry.

Although to the experimentalist, the problem of 'intermolecular' forces is dominated by the properties of the rare gases, the theoretician is less inhibited, so that the volume includes discussion not only of the Argon-Argon potential but also (H₂O)₂ and even an extensive section on DNA.

The volume contains only four chapters; these are by no means equal in weight, being set out more in the manner of a dinner menu. As an hors d'oeuvres we have a review of the basic theory by A. D. Buckingham. This chapter includes applications to small molecules and is a clear summary which will be welcomed by students as well as active researchers in the field.

The main course which follows, occupies well over 200 pages and is Claverie's elaboration of the approximate formulae for the interactions between large molecules. It is by no means an easy read but includes a detailed account of the theoretical foundation and derivation of formulae. This chapter gives ample reference to applications but less actual examples, so that one is left wondering just how well the theory actually stands up in practice.

The sweet, after a heavy main course, is as enjoyable as such things should be: the principles of nucleic acid structure and function from the point of view of constituent interactions, by Rein. There is no doubt about the fascination or importance of this topic, but one is left far from totally convinced about the quality and value of the theoretical calculations described. Theory makes predictions which are "within reason", or comparison with experiment is "somewhat favourable". Difficult problems such as the influence of solvent are not avoided; nor are they solved. The biological inferences are appealing but there still seems to be some way to go before computational methods are an essential contributor to understanding.

The savoury course to complete the experience is a chapter by Schuster on the hydrogen bond. This is a useful updating of the many reviews on this important topic. Indeed, so central is this aspect of interaction that all four chapters contain sections on hydrogen bonding. Alternatively this could be viewed as an over-light application of the editorial hand, as separately suggested by a referencing system which is not consistent among the four chapters. As hydrogen bonds are taken so seriously, it is a pity that a book published in 1978 makes no mention of the important experimental study of the water dimer by electric resonance spectroscopy published by Dyke *et al.* in the spring of 1977. These results are slightly uncomfortable for the theoretician, and had they been to hand for the authors, they might have been more guarded in their optimism.

The final impression is of a subject in which much is left to do, but the problems are by no means intractable: an attractive research situation and one for which this book will serve as an admirable basis.

Graham Richards

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Broad survey of teratology

Handbook of Teratology. Edited by J. G. Wilson and F. Clarke Fraser. Vol. 1: Pp. 476; vol. 2: Pp. 491. (Plenum: New York and London, 1977.) \$54 each volume.

Two prestigious investigators have undertaken a monumental task of editing as well as contributing to this authoritative new treatise in four volumes. The first two volumes on "General Principles and Etiology" and on "Mechanisms and Pathogenesis" have now appeared. A third volume on "Comparative Maternal and Epidemiologic Aspects" has just been published, and a fourth and final volume on "Research Procedures and Data Analysis" is yet to come.

These first two volumes, with no fewer than 30 contributors to twenty-seven elegant chapters, cover the aspects of teratology with a wealth of published and original data and very full and useful bibliographies. After a fascinating review of teratological history by Joseph Warkany, Wilson in his usual concise and common-sense style places our knowledge of the teratogenicity of drugs and environmental chemicals into perspective in three profusely referenced chapters. Clarke Fraser has written an excellent review chapter on the relationship between animal studies to the problem in man, which should be read by anybody planning to embark on teratological surveillance or research.

Brent provides a good summary of mutagenicity and teratogenicity of radiation and other physical agents. The discussions of the role of nutritional deficiencies by Lucille Hurley, of atmospheric gases by Gobovski and of extremes of temperature by Edwards and Warner, point to neglected areas of research. The whole section on examples of abnormal organogenesis, consisting of seven chapters of volume two together with the chapter on time-position relationships with particular reference to cleft lip and cleft palate, by Daphne Transler and Clarke Fraser, are well written and well illustrated, and provide concise and authoritative summaries of the subject, together with good bibliographies.

An all too brief review of the enormous subject of the action of mutagenic agents has been included with valuable space being devoted to lists of chemicals, agricultural agents, industrial compounds, feed and feeding additives, and so on; but there has been no real attempt at an evaluation of the dangers. The chapter on maternal and endocrine imbalances, an important subject today, is lamentably brief and surprisingly omits any discussion of sexual ambiguity produced by maternal 'hormone' ingestion or endo-

crine abnormalities. The one on gene expression is also extremely brief and would certainly benefit from more elaboration. The section on numerical and structure chromosome abnormalities is a repetitive review of clinical entities, information about which might better be obtained from a reference book on that subject alone. This section also lacks an in-depth discussion of the mechanisms producing chromosome aberrations which should be its rightful place in this treatise.

The problems inherent in the creation of this type of treatise are only too well illustrated by the handbook. The numerous authors, each an established scientist and generally a leader in the field, tend to make for excessive repetition, and in this instance repetitious discussion of drug effects. These volumes suffer from a

certain lack of consistency and continuity; the reading of such a treatise would be enhanced by fewer contributing authors and a consolidation of chapters in order to present more in-depth material regarding, for example, techniques pertinent to that chapter. Nonetheless, Wilson and Fraser are to be commended for their efforts, very largely successful, to provide a summary of a mass of information. The handbook will be a useful addition to the library, particularly for those interested in a broad survey of teratology and a guide to the relevant literature.

K. M. Laurence

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Reproductive strategies in langurs

The Langurs of Abu: Female and Male Strategies of Reproduction. By Sarah Hrdy. Pp. 361. (Harvard University Press: London and Cambridge, Massachusetts, 1978.) £12.25; \$17.50.

"FROM his high vantage point, Mug strikes a sentinel's pose and stares off into the distance. Then, abruptly, the stocky gray form descends from his rooftop perch, charges directly at Itch, and grabs at the infant clinging to her belly. Itch whirls to face Mug, plants her front paws as she lunges at him, grimaces, and bares her teeth. Within seconds of this assault, Old Sol, the one-armed female Pawless, and a third, unidentified female, join in the counterattack. The three defenders interpose themselves between Mug and Itch, lunge at the male, and chase him up a tree. In the wake of this assault, a trembling Itch is left alone to hold her infant, Scratch, who is spattered with flecks of blood. Here was the phenomenon, the bizarre Dharwar aberration of adult males attacking infants, that had brought me to India to study langurs..."

It is commonly supposed that murder is a uniquely human event. When a Japanese primatologist, Yukimaru Sugiyama first described systematic killing of infants by male langurs in 1965, the phenomenon was widely explained by others as a pathological product of high population density. Sarah Hrdy's book (from which the above extract is taken) provides convincing evidence that this is not the case and that infanticide permits males which have recently established hegemony over a breeding unit to maximize their reproductive success.

Female langurs live in harem groups including, on average, about a dozen

mature females and their offspring plus one, or sometimes two, mature males. Young males leave their mothers' groups to join all male bands whose ranges overlap those of several breeding groups. There is evidently strong competition between males for possession of harems; and from time to time male bands invade breeding groups, expelling the resident male. The invading males then leave, or are driven out by the strongest animal, until (in most cases) only one remains. Successful invaders systematically kill infants, a strategy which serves to bring females quickly into oestrus, thus allowing the male to increase his reproductive success. Further evidence strongly supports this interpretation of infanticide: resident males never kill the infants which they themselves have sired, and similar events have now been described in a wide variety of other species which live in harem-type groups when resident males are replaced by outsiders. As might be expected, females attempt to prevent their infants being killed. Of particular interest here are the cooperative efforts of older females to rescue infants attacked by males. As Hrdy points out, they probably increase their inclusive fitness by doing so, as females belonging to the same troop are closely related.

The book covers several other aspects of social behaviour and makes extensive and imaginative use of modern evolutionary theory. It is written with wit, humour and an almost complete lack of formal statistics. As a result it is easy to read, but firm results based on adequate observation samples are sometimes difficult to disentangle from suggestions relying on selected anecdotes.

T. H. Clutton-Brock

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Applications of isoelectric focusing

Biological and Biomedical Applications of Isoelectric Focusing. Edited by N. Catsimopoulos and J. Drysdale. (Plenum: New York, 1977.) \$39.

ANY collection of the applications of a specialised technique in science is open to two criticisms: firstly that it inherently only appeals to a small group of readers, as the bulk of the applications will be of little interest to them; and secondly that such collections are liable to be outdated even before publication. The editors of this book have almost avoided these pitfalls, the first by selecting a subject which is of wide application and therefore of general interest in protein chemistry, and the second by choosing a technique the methodology of which has changed little in the past six years. They are thus able virtually to ignore the basic techniques of isoelectric focusing, except in so far as it is introduced by the individual authors.

Chapters 1, 2, 5 and 10 will be of special interest to the clinical chemist, as they deal with the analysis of the body fluids using isoelectric focusing, with special emphasis on the haemoglobins and immunoglobins in chapters 2 and 5. Chapter 5 is particularly valuable, as the technique of isoelectric focusing has made fundamental contributions to the problems of antibody heterogeneity and to the study of antibody synthesis, both *in vivo* and *in vitro*. Hoffman provides an excellent, up-to-date account of this subject. Latner (chapter 10) gives a very complete review of the applications of his two-dimensional isoelectric focusing-gel electrophoresis technique to serum proteins in normal and pathological conditions. Although two-dimensional methods are notoriously difficult to reproduce, the complexity of the protein components in serum necessitates this approach, and Latner's illustrations illustrate the value of the method in clinical chemistry.

More specialised chapters are contributed by Florini on muscle proteins and by Schmidt-Ullrich and Wallach on membrane proteins. Both these groups are notorious for solubility and aggregation problems which may necessitate the use of strong urea, and/or detergent solutions, both for extraction and for fractionation. Both reviewers cover these points in detail and offer methods for their solution.

The application of isoelectric focusing in plant biochemistry is well illustrated by Wrigley's chapter 8 on seed proteins. Here again, the number

of protein components present in crude extracts has necessitated the development of two-dimensional methods both for species identification and for genetic studies. The lectins, a class of plant proteins that are becoming increasingly important as carbohydrate-specific biochemical reagents, are also covered in this review, which makes refreshing reading for the more classically minded biochemist.

I found MacGillivray's review on the use of isoelectric focusing in the separation and characterisation of nuclear non-histone proteins particularly interesting and instructive. It provides an excellent example of the way in which sophisticated analytical electrophoretic methods can be used to monitor and design preparative methods for the isolation of even minor components of complex mixtures. The components themselves are

often intractable in nature, and available only in small quantities, but they may nevertheless be of key importance in gene expression.

While concluding that this is very often a valuable source book, may I plead for a moratorium on books on isoelectric focusing. At least one major volume has been devoted to this relatively small field every year since 1973, and the subject matter is getting very well-worn. In any case revolutionary new advances in preparative isoelectric focusing are imminent, and it would be well to wait for these to be incorporated into the literature, before attempting further surveys.

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Astro-art

The New Challenge of the Stars: A Science Fact Look at Science Fiction. By Patrick Moore and David Hardy. Foreword by Arthur C. Clarke. Pp. 62. (Mitchell Beazley: London, 1978.) £4.95.

ASTRONOMY and art are frequent bedfellows. The astro-artists, with brush, palette and Allen's *Astrophysical Quantities* to hand, can easily transcend the barriers of time and space, leaving the astronomer and astrophysicist shackled in the present and to the immediate vicinity of the Sun. He can paint what the eyes of mankind can never see. We can be transported to the birth of the Solar System, to the centre of a globular cluster, to the edge of a supernova explosion. The end-products are paintings of considerable vibrance, colour and beauty but more than that they are paintings of considerable educative worth. The artist molds his scene to embody all the factual information available at the time. The scientist and scholar, as well as enjoying the paintings, can use them as vivid images of present-day knowledge, an easily assimilable set of stepping stones to future theories and hypotheses.

The New Challenge of the Stars is a large format book (23 × 33 cm) and contains 30 or so full-page to double-page reproductions of David Hardy's space paintings. Half the paintings are space fact, my favourites being "An unmanned probe re-appears after having passed through the coma of the comet"—the ghostly form of the comet illuminating the Mariner-type spacecraft with the crescent Earth and Moon hovering in the distance; "Saturn as seen from Rhea"—Rhea assumed to have a Moon-like surface being completely menaced by the leaden domineering planet with its edge

on rings slicing razor sharp across the sky; and "Icarus"—barren and cratered, diffused in the red glow of Mars with the Sun ominously emerging from eclipse.

The remainder of the pictures are from the realms of science fiction and fantasy. We see a crippled spacecraft lying like a wounded snake across the surface of a planet, crawling with robot repair vehicles, the sky behind filled with the splendour of a giant star pouring plasma into a black hole; we have asteroids hollowed out to produce space Arks, space colonies being attacked by alien civilisations, the desolation of futuristic cities after the nova explosion of their stellar suns, lunar and Martian bases, and embryonic space towns orbiting Earth.

Patrick Moore's text is business-like and informative and geared to the inquisitiveness of the amateur astronomer. However, the meat of this book is the artwork. It is magnificent and David Hardy will enthuse and enchant today's young astronomers in exactly the way that the previous generation marvelled at the paintings of Chesley Bonestell in *The Conquest of Space* (text by Willy Ley; Sidgwick and Jackson: London, 1950) and of R. A. Smith in *Exploration of the Moon* (text by Arthur C. Clarke; Frederick Muller: London, 1954).

Astro-art has been with us a long time; this book and the ever increasing popularity of the American monthly magazine *Astronomy* show that it is in an extremely healthy state today. In the future we will realise that some of Hardy's visions are incorrect. But the enjoyment and wonder they engender today in the minds of young readers will have played a great part in motivating them to strive for more knowledge, knowledge which will possibly prove him wrong.

David W. Hughes

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